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Chemical Compositions and Cytotoxic Activities of Leaf Essential Oils of Four Lauraceae Tree Species from Monteverde, Costa Rica

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Abstract: The leaf essential oils of four members of the Lauraceae *Licaria excelsa*, *Licaria triandra*, *Persea schiedeana*, and *Rhodostemonodaphne kunthiana*, from Monteverde, Costa Rica, were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The leaf oil of *L. excelsa* was dominated by the monoterpenes α-pinene (42.9%), β-pinene (22.0%) and myrcene (17.2%), while *L. triandra* was also rich in pinenes (40.9% and 28.5%, respectively). *Persea schiedeana* had considerable amounts of the sesquiterpenes δ-cadinene (18.5%), α-copaene (15.1%), and (*E*)-caryophyllene (13.3%). *Rhodostemonodaphne kunthiana* leaf oil had germacrene D (64.4%) and bicyclogermacrene (17.6%) as the major components. The leaf essential oils were screened for *in-vitro* cytotoxic activity against MDA-MB-231 and Hs 578T human tumor cells. *R. kunthiana* leaf oil showed notable activity against MDA-MB-231.

Keywords: *Licaria excelsa*; *Licaria triandra*; *Persea schiedeana*; *Rhodostemonodaphne kunthiana*; essential oil composition; Lauraceae; α-pinene; β-pinene; δ-cadinene; germacrene-D; bicyclogermacrene

1. Introduction

The Laurel family (Lauraceae) of plants is composed of 52 genera and nearly 3000 species, mainly distributed in tropical and warm subtropical regions [1]. Members of the family have a wide variety of uses ranging from spices to drugs, which make them a valuable economical resource [2]. This work presents the leaf essential oil compositions for four members of the Lauraceae from

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Monteverde, Costa Rica: *Licaria excelsa* Kosterm., *Licaria triandra* (Sw.) Kosterm., *Persea schiedeana* Nees, and *Rhodostemonodaphne kunthiana* (Nees) Rohwer. Leaf oil compositions of *L. triandra* grown in Cuba [3] and *P. schiedeana* grown in California [4] have been previously reported, but to our knowledge, the essential oil compositions of *L. excelsa* and *R. kunthiana* have not been investigated.

2. Materials and Methods

2.1. Plant Material

Leaves of *L. excelsa*, *L. triandra*, *P. schiedeana*, and *R. kunthiana*, were collected from mature trees in the Monteverde region of Costa Rica. The plants were identified by W. A. Haber. Voucher specimens have been deposited in the herbarium of the Missouri Botanical Garden and the National Herbarium of Costa Rica. The fresh leaves were chopped and hydrodistilled for four hours with concomitant extraction with $CHCl_3$ using a Likens-Nickerson apparatus to give the essential oils (Table 1).

Table 1. Collection and hydrodistillation of leaves from Lauraceae from Monteverde, Costa Rica.

Plant	Voucher number	Collection site (date)	Mass of fresh leaves, g	Yield of essential oil, mg (% yield)
Licaria excelsa	Haber 11091	Monteverde community[a] (5-23-05)	50.9	73.8 (0.145%)
Licaria triandra	Haber 8432	Monteverde community[a] (5-14-08)	50.3	363.4 (0.722%)
Persea schiedeana	Haber 8185	Monteverde Cloud Forest Preserve[b] (5-6-08)	65.4	461.9 (0.706%)
Rhodostemonodaphne kunthiana	Haber and Cruz 6791	Peñas Blancas Valley[c] (5-14-08)	34.0	127.3 (0.374%)

[a]Monteverde community (10.3059 N, 84.8144 W, 1380 m above sea level).
[b]Monteverde Cloud Forest Preserve (10.3483 N, 84.7633 W, 1530 m above sea level).
[c]Peñas Blancas Valley (10.3091 N, 84.7162 W, 800 m above sea level).

2.2 Gas Chromatography-Mass Spectrometry

The leaf essential oils were subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector (EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 8.23 psi and flow rate of 1.0 mL/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 60°C initial temperature, hold for 5 min; increased at 3°/min to 280°C. The sample was dissolved in $CHCl_3$ to give a 1% w/v solution; 1 µL injections using a splitless injection technique were used. Identification of oil components was achieved based on their retention indices (RI, determined with reference to a homologous series of

normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [5] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

Table 2. Chemical compositions of Lauraceae leaf oils from Monteverde, Costa Rica.

RI	Compound	Percent Composition			
		L. excelsa	***L. triandra***	***P. schiedeana***	***R. kunthiana***
940	α-Pinene	42.9	40.9	0.1	---
953	Camphene	2.6	4.8	---	---
979	β-Pinene	22.0	28.5	t[a]	---
993	Myrcene	17.2	0.6	---	---
1005	α-Phellandrene	---	t	---	---
1010	δ-3-Carene	---	t	---	---
1020	*p*-Cymene	---	t	---	---
1025	*o*-Cymene	---	0.8	---	---
1031	Limonene	0.9	2.2	---	---
1034	1,8-Cineole	2.7	t	---	---
1061	γ-Terpinene	---	t	---	---
1090	Terpinolene	---	t	---	---
1101	Linalool	0.8	---	---	---
1334	δ-Elemene	---	---	0.7	---
1349	α-Cubebene	---	0.4	1.5	t
1376	α-Copaene	---	0.6	15.1	0.9
1384	β-Bourbonene	0.2	t	t	0.3
1391	β-Cubebene	---	1.1	t	t
1392	β-Elemene	0.2	---	2.1	1.7
1394	7-*epi*-Sesquithujene	---	t	---	---
1419	(*E*)-Caryophyllene	3.0	6.5	13.3	1.1
1428	β-Copaene	---	t	0.4	0.3
1435	γ-Elemene	---	t	t	---
1436	α-*trans*-Bergamotene	---	---	0.7	---
1442	Aromadendrene	---	0.1	---	t
1443	6,9-Guaiadiene	---	---	t	---
1450	*cis*-Muurola-3,5-diene	---	---	1.0	---
1453	α-Humulene	0.8	1.5	1.8	t
1459	(*E*)-β-Farnesene	0.2	---	---	---
1459	Alloaromadendrene	---	t	0.6	1.0
1461	*cis*-Cadina-1(6),4-diene	---	t	t	---
1473	*trans*-Cadina-1(6),4-diene	---	t	3.1	---
1477	γ-Muurolene	---	t	---	---
1479	α-Amorphene	---	---	3.1	---
1482	Germacrene D	1.5	1.7	4.5	64.4
1487	β-Selinene	---	0.9	1.8	---
1493	*trans*-Muurola-4(14),5-diene	---	0.2	1.0	0.3
1495	Unidentified[b]	1.2	---	---	---
1496	*cis*-β-Guaiene	---	---	2.7	---
1498	γ-Amorphene	---	2.2	---	---
1498	α-Zingiberene	---	---	3.4	---
1499	Bicyclogermacrene	---	---	---	17.6
1501	α-Muurolene	0.3	t	2.0	---
1509	δ-Amorphene	---	---	t	0.7

RI	Compound	Percent Composition			
		L. excelsa	*L. triandra*	*P. schiedeana*	*R. kunthiana*
1514	β-Curcumene	---	t	---	---
1515	γ-Cadinene	1.1	0.9	2.7	1.1
1524	δ-Cadinene	1.3	0.9	18.5	2.5
1533	*trans*-Cadina-1,4-diene	---	0.1	2.5	t
1538	α-Cadinene	---	t	0.8	t
1546	*cis*-Sesquisabinene hydrate	---	0.5	---	---
1556	Germacrene B	---	---	1.2	t
1568	(*E*)-Nerolidol	---	0.6	---	---
1580	Spathulenol	---	0.8	---	4.9
1582	Unidentified[c]	---	---	0.8	---
1583	Caryophyllene oxide	---	1.3	---	0.3
1600	Guaiol	---	---	---	0.6
1607	Humulene epoxide II	---	0.1	---	---
1627	1-*epi*-Cubenol	---	0.2	2.1	---
1639	Muurola-4,10(14)-dien-1β-ol	---	---	---	0.3
1640	τ-Cadinol	0.7	0.4	5.4	0.5
1644	α-Muurolol	---	---	1.1	---
1646	Unidentified[d]	---	---	0.9	---
1650	α-Eudesmol	---	0.8	---	---
1653	α-Cadinol	0.4	---	5.0	1.5
1654	Valerianol	---	0.4	---	---
	Total identified	98.8	100.0	98.3	100.0
	Monoterpene hydrocarbons	85.7	77.7	0.1	0.0
	Oxygenated monoterpenoids	3.5	0.0	0.0	0.0
	Sesquiterpene hydrocarbons	9.8	17.2	84.5	91.9
	Oxygenated sesquiterpenoids	1.1	5.1	15.3	8.1

[a]t = trace (< 0.05%)

[b]MS: *m/z* = 202(26%), 183(26%), 159(61%), 145(35%), 132(41%), 121(66%), 119(72%), 105(89%), 91(100%), 79(46%), 77(47%), 69(25%), 67(28%), 55(38%).

[c]MS: *m/z* = 204(63%), 189(47%), 161(100%), 147(52%), 133(46%), 119(100%), 107(63%), 105(93%), 93(63%), 91(97%), 81(45%), 79(54%), 77(48%), 69(35%), 67(36%), 55(39%).

[d]MS: *m/z* = 204(40%), 189(12%), 161(100%), 119(51%), 105(62%), 95(22%), 93(26%), 91(22%), 81(21%), 79(25%), 77(21%), 69(10%), 67(10%), 55(12%).

2.3 Cytotoxicity Screening

In-vitro cytotoxic activity against MDA-MB-231 (ATCC No. HTB-26) and Hs 578T (ATCC No. HTB-126) cells was carried out using the MTT method for cell viability as previously described [6]. Cytotoxic activities of the leaf oils are summarized in Table 3.

Table 3. Cytotoxic activities of essential oils and components.

Material	Cytotoxicity	
	MDA-MB-231	Hs 578T
Licaria excelsa	0.0[a]	0.0[a]
Licaria triandra	25.0(15.6)[a]	8.8(6.1)[a]
Persea schiedeana	0.0[a]	0.0[a]
Rhodostemonodaphne kunthiana	97.8(2.2)[a]	0.0[a]
α-Pinene	> 100[b]	> 100[b]
β-Pinene	> 100[b]	> 100[b]
(*E*)-Caryophyllene	31.6(0.2)[b]	78.3(8.3)[b]
Germacrene D	54.2(5.7)[b]	55.2(5.3)[b]
Doxorubicin[c]	25.9(2.4)[b]	10.4(1.2)[b]

[a] % kill at 100 μg/mL (standard deviations in parentheses).
[b] LC_{50}, μg/mL (standard deviations in parentheses).
[c] Positive control.

3. Results and Discussion

The chemical compositions of *L. excelsa*, *L. triandra*, *P. schiedeana*, and *R. kunthiana* leaf oils are summarized in Table 2. Both *L. excelsa* and *L. triandra* leaf oils showed comparable chemical compositions and were dominated by monoterpene hydrocarbons, especially α- and β-pinene. The specimen of *L. triandra* collected from Cuba had a similar chemical composition, but selin-11-en-4α-ol (15.1%) was reported as the most abundant compound in the sample [3], which was absent in the sample from Costa Rica. Different environmental conditions such as the season, altitude, and climate factor into the growth of a plant, and may account for the differences between *L. triandra* collected in Cuba versus Costa Rica.

The leaf essential oils of *P. schiedeana* and *R. kunthiana* were dominated by sesquiterpenoids. *P. schiedeana* showed significant amounts of δ-cadinene (18.5%), α-copaene (15.1%), and (*E*)-caryophyllene (13.3%) along with small amounts of τ-cadinol (= *epi*-α-cadinol, 5.4%), α-cadinol (5%), and germacrene D (4.5%). An older previous analysis of *P. schiedeana* grown in California showed a qualitatively different chemical profile, with abundant β-pinene (16%) and estragole (7%) [4] *P. americana* (the avocado) shows two predominant chemotypes, an estragole-rich chemotype and an (*E*)-caryophyllene-rich chemytype [4,7]. *P. caerulea* [8] and *P. indica* [2] leaf oils are rich in (*E*)-caryophyllene and germacrene D. The leaf essential oils of *P. borbonia*, *P. humilis*, and *P. palustris*, by contrast, are dominated by oxygenated monoterpenoids, 1,8-cineole and camphor [9]. The percent composition of *R. kunthiana* revealed a notable amount of germacrene D (64.4%) and bicyclogermacrene (17.6%). These sesquiterpenes have also been found to be abundant components of *Beilschmiedia* [10] and *Ocotea* [11] leaf essential oils. None of the essential oils showed *in-vitro* cytotoxic activity against Hs 578T human breast ductal carcinoma cells, but *R. kunthiana* leaf oil was cytotoxic toward MDA-MB-231 human breast adenocarcinoma cells. The cytotoxicity is likely due to the high concentrations of sesquiterpene hydrocarbons, particularly germacrene D, which does show cytotoxic activity on this cell line.

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References

[1] D.J. Mabberley (1997). The Plant-Book. Cambridge University Press, Cambridge, UK.
[2] P. Weyerstahl, H. Marschall and R.W. Scora (1993). Constitients of the leaf essential oil of *Persea indica* (L.) K. Spreng. *Flavour Fragr. J.* **8**, 201-207.
[3] J. Pino, R. Marbot, A. Payo, D. Chao, P. Herrera and M.P. Martí (2005). Leaf oil of *Licaria triandra* (Sw.) Kostermans, *J. Essent. Oil Res* **17**, 382-383.
[4] B.O. Bergh, R.W. Scora and W.B. Storey (1973). A comparison of leaf terpenes in *Persea* subgenus *Persea*. *Bot. Gaz.* **134**, 130-134.
[5] R.P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectrometry, 4th Ed. Allured Publishing Corporation. Carol Stream, Illinois.
[6] D.M. Moriarity, A. Bansal, R.A. Cole, S. Takaku, W.A. Haber and W.N. Setzer (2007). Selective cytotoxic activities of leaf essential oils from Monteverde, Costa Rica. *Nat. Prod. Commun.* **2**, 1263-1268.
[7] J.A. Pino, R. Marbot and M.P. Martí (2006). Leaf oil of *Persea americana* Mill. var. *drymifolia* cv. Duke grown in Cuba. *J. Essent. Oil Res.* **18**, 440-442.
[8] W.N. Setzer, S.L. Stokes, A.F. Penton, S. Takaku, W.A. Haber, E. Hansell, C.R. Caffrey and J.H. McKerrow (2007). Cruzain inhibitory activity of leaf essential oils of Neotropical Lauraceae and essential oil components. *Nat. Prod. Commun.* **2**, 1203-1210.
[9] A.O. Tucker, M.J. Maciarello, B.E. Wofford and W.M. Dennis (1997). Volatile leaf oils of *Persea borbonia* (L.) Spreng., *P. humilis* Nash, and *P. palustris* (Raf.) Sarg. (Lauraceae) of North America. *J. Essent. Oil Res.* **9**, 209-211.
[10] W.A. Haber, and W.N. Setzer (2007). Leaf essential oil composition of five species of *Beilschmiedia* from Monteverde, Costa Rica, *Nat. Prod. Commun.* **2**,79-83.
[11] S. Takaku, W.A. Haber and W.Setzer (2007). Leaf essential oil compostion of 10 Species of *Ocotea* (Lauraceae) from Monteverde, Costa Rica, *Biochem. System. Ecol.* **35**, 525-532.