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Exploring the chemical composition of volatile leaf oils from *Illicium ekmanii* and *I. hottense*, endangered species endemic to Hispaniola

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Abstract
This study reports the foliar oil composition of *I.ekmanii* from sites across two mountain ranges in the Dominican Republic and for *I.hottense* from Les-Cayes, Haiti. We used Clevenger hydro-distillation for extraction, GC-FID for quantification, and GC-MS, MS spectral libraries, and LRIs for identification. All of the *Illicium* samples in this study showed the presence of linalool (3.22-17.15%), α-terpineol (0.41-1.99%), α-cubebene (0.19-0.65%), α-copaene (4.03-26.99%), β-elemene (0.26-0.93%), β-caryophyllene (5.43-35.70%), α-humulene (0.86-3.53%), δ-cadinol (0.2-0.79%), and τ-muurolol (0.92-4.33%). *I. hottense* leaf oil was found to be sesquiterpene and oxygenated monoterpene-rich and was characterized by copious relative amounts of β-caryophyllene (35.70 ± 0.265% w/w), linalool (17.15 ± 0.215% w/w), and cis-methyl eugenol (12.08 ± 0.09% w/w), three compounds that made up 65% of the oil composition. *I. ekmanii* from the Cordillera Central range showed sesquiterpene-rich oils with an abundance of β-caryophyllene and α-copaene. In contrast, a sample from the Septentrional range showed an abundance of phenylpropenes in Salcedo, while another showed trace phenylpropenes and was sesquiterpene-rich in Isabelle Torres. Additional research into the phenylpropene-rich samples found in the Septentrional Range is warranted to determine if this essential oil composition is a chemotypical difference, an environmental difference, and/or warrants further taxonomical/molecular examination.

Keywords: Essential oil, *Illicium*, sesquiterpene, schisandraceae, hispaniola

1. Introduction
The *Illicium* genus (Schisandraceae) consists of 40 - 50 species of understory, woody shrubs and trees distributed in Southeast Asia, the south-eastern United States, Mexico and the Greater Antilles [1]. The genus possesses a disjunct distribution with distinct Old World and New World clades [1, 2]. Research into the phytochemical composition of *Illicium* has shown the genus to produce many unique and biologically active secondary metabolites [3]. These compounds have been shown to have value as flavoring components [4], are used medicinally [5-8], possess antioxidative [9], insecticidal [3], antimicrobial [10], and fungicidal activities [11, 12] and, cumulatively, these studies have elucidated the chemical diversity within the genus. The quantities and qualities of various essential oils in the species can vary depending on time of harvest, seasonal and climactic conditions, geographic location and geology, freshness of the sample and drying conditions, subspecies variation and extraction technique [13,14].
Previous studies on *Illicium* essential oils have suggested there are three groups of chemotypes: phenylpropene-rich chemotypes, phenylpropene and oxygenated monoterpene chemotypes, and sesquiterpene dominated chemotypes [15]. In *I. verum*, (E)-anethole is often reported to be the most abundant compound in its phenylpropene dominated oils, ranging from 7.9% to 93.9% [4, 13, 16]. *I. grifffithii* reports from Vietnam show phenylpropene and oxygenated monoterpene-rich oils with 51.6 - 65.3% safrole in the aerial portions of the plant [17] and in the fruit, linalool (19.6%), p-methoxy phenyl acetone (11.8%), terpinen-4-ol (11.0%), limonene (10.6%) and safrole (10.1%) were shown to dominate [18]. Examples of the sesquiterpene-rich chemotypes include *I. simonsii* [19] and *I. majus* [20], where the fruit and pericarps, respectively, were dominated by β-caryophyllene.
Illicium ekmanii A.C. Smith and *I. hottense* A. Guerrero, Judd & Morris are aromatic plant species found in the mountainous regions of Hispaniola, grow in threatened cloud-forest and ombrophile forest ecosystems, and represent two of the lesser-known New World species [21]. These species, both of which are endemic to the island [2, 22, 23], are classified as endangered and

vulnerable, according to the Red List of Vascular Flora in the Dominican Republic [24] and the International Union for Conservation of Nature [25]. They are mainly found in the Cordillera Septentrional and Cordillera Central mountain ranges in the Dominican Republic and the Massif de la Hotte range in Haiti (Figure 1).

Little is known regarding the composition of volatile oils produced in species of New World *Illicium*, with only one study of *I. floridanum* and *I. parviflorum* showing them to be sesquiterpene-rich and phenylpropene-rich chemotypes, respectively [26]. *I. parviflorum* is notable for showing nearly 70% safrole, a compound also found in Old World species like *I. griffithii*, with many important medicinal uses [27], psychoactive properties and toxicity [28, 29]. The aim of the present study, therefore, is to advance on what is known about New World chemotypical expressions, by providing a detailed chemical composition analyses for the leaves of *I. ekmanii* and *hottense*, and to look within various populations of *I. ekmanii* from the northernmost Septentrional and the Cordillera Central mountain ranges of Hispaniola, in an effort to identify the resident chemotypes.

2.1 Materials and Methods

2.1 Sampling Sites

For *I. ekmanii*, sampling sites were determined systematically using herbarium and literature searches in conjunction with geospatial analyses and predictive modelling to determine the current and estimated occurrence sites of *I. ekmanii*. The locales with the highest likelihood were scouted and the identity of populations confirmed (Figure 1). This study present results for *I. ekmanii* samples collected within the Dominican scientific reserves of La Salcedoa (CSSAL), and Isabel de Torres (CSIT) in the Cordillera Septentrional, and from Loma Miranda (CCLM), Cerro Angola (CCCA), and El Firme de Juan Adrián (CCFJA) in the Cordillera Central. The information regarding herbarium deposition samples for each site are shown in Table A.

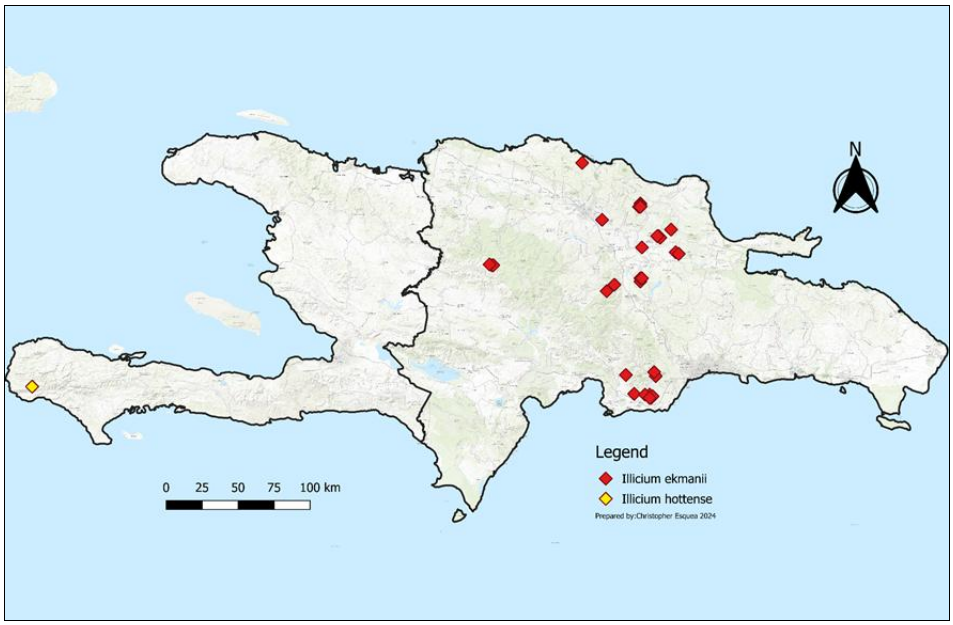

Fig 1: Distribution of confirmed I. ekmanii (red) and I. hottense (orange) populations on Hispaniola.

For *I. hottense* (HOT), samples were collected during a survey expedition for multiple species from the Grand-Bois locality in the Sur department, Les-Cayes Province at an elevation of 1082 m. All of the sampling sites are ecologically fragmented and *Illicium* populations are threatened due to various land-use pressures and other factors (e.g. mining and development), so the exact coordinates are withheld in this report and we refer only to locality names throughout.

Table 1: Sampling site information and Jardin Botanico Santo Domingo (JBSD) voucher information.

Sample ID	Sample Collection Date	Herbarium Voucher	Location/Locality	Elevation (m)
CCLM	18 March 2018	Teodoro Clase #10428	Firme Loma Miranda	698
CCFJA	17 March 2019	Teodoro Clase #11125	Firme Juan Adrian	1112
CCCA	21 Nov 2021	Teodoro Clase #7164	Cerro Angola	1500
CSSAL	10 Jan 2019	Rosa Rodriguez #382	La Jibara	635
CSIT	8 Dec 2020	Teodoro Clase #7372	Loma Isabel de Torres	770
HOT	21 July 2019	Teodoro Clase #11646	Les-Cayes	1082

2.2 Plant Materials

Varied amounts of leaves (< 100 g) of *I. ekmanii* and *I. hottense* were collected from healthy plants using conservation-based sampling techniques. Leaf samples were selected from a minimum of five individual plants and voucher specimens were deposited in the herbarium of the National Botanical Garden in Santo Domingo (JBSD), under the voucher numbers shown in Table A. Leaf samples were homogenized and dried in the shade for about two-weeks then stored at 3 °C until shipping. Samples were inspected by a plant pathologist at the Ministry of Agriculture (Dominican Republic) for phytosanitary certification and then shipped for analytical testing and phytochemical characterization. In the laboratory, samples were stored at -20 °C until extraction.

2.3 Extraction

The essential oil was extracted from 5-10 g of dried leaves by hydrodistillation for 5-hours using a Clevenger apparatus. The

distillate was washed with duplicate additions of 50 mL hexane (HPLC grade; Fisher Scientific). The aqueous layer was discarded and the hexane solution was dried over anhydrous sodium sulphate. Hexane was removed using a vacuum rotary evaporator at 50 °C. The resulting oils were transferred to a pre-weighed amber glass vial, sealed, and kept in the dark at 2 °C prior to analysis. Oil yield was calculated as mean percent weight from dry leaves from triplicate extractions for CCLM, CSSAL, and HOT, and on one extraction for CCFJA, CCCA, and CSIT, as not enough material was available for triplicate extractions at the time of collection.

2.4 GC Analyses

GC analyses were performed on each triplicate extraction for CCLM, CCSAL, and HOT. Triplicate analytical replicates were performed on the single extractions for CCFJA, CCCA, and CSIT. GC/MS was performed on a Shimadzu GCMS-QP2020 system equipped with a Shimadzu SH-Rxi-5MS 30 m x 0.25 mm capillary column containing a 0.25 µm 5% diphenyl / 95% dimethyl polysiloxane stationary phase. A volume of 0.5 µL of a 1:100 (v/v) dilution of the EO in HPLC grade hexane (Fisher Scientific) was injected into the split injector using a split ratio of 20 and pure helium as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 250 °C and the gradient temperature program started at 60 °C for 5 minutes, then was increased at 3.0 °C/min to 250 °C, and held for 5 minutes. The total runtime was 73.33 minutes. The MS transfer line was held at a temperature of 280 °C and the MS electron ionization source was held at 250 °C. After a 3-minute solvent delay, MS scans were performed from 40 to 450 m/z in full scan mode using an EI source.

The chemical constituents were identified based on comparison of the linear retention indices (LRI) calculated relative to a series of n-alkanes (C7-C40; Sigma-Aldrich), confirmed by comparison with published LRI values [30,31], and via mass spectral comparison using the 2014 NIST mass spectral library, accessed via the Shimadzu Lab Solutions software version 4.45 [32].

Relative amounts of each compound were calculated on the basis of peak area ratios from GC-FID results obtained on the same Shimadzu QP2020 system equipped with a flame ionization detector and operating under the same conditions as described above in the GC-MS temperature programing section. The FID was operated at a detector temperature of 250 °C with a constant makeup flow of 30 mL/min helium, 40 mL/min hydrogen, and 400 mL/min analytical grade air. No correction factor was applied to relative areas.

3. Results and Discussion

3.1 Foliar oil yield

The leaf essential oil content for the five *I. ekmanii* sites ranged from 0.14 - 1.11% w/w while *I. hottense* yielded 0.85 ± 0.21% oil w/w (lower portion of Table B). The oils appeared colorless and had a straw odor, with the exception of the high yielding sample, CSSAL, which had a sweet and spicy odor and a slight yellow color. By comparison, reported yields from *I. verum* in Asia showed results of 0.01-0.60% oil in the leaves [33]. The more closely related *I. floridanum* and *I. parviflorum* have previously been reported to have 0.07 and 0.35% w/w oil in the leaves, respectively [26], values which fall within the range of those we have presently observed for *I. ekmanii* and *I. hottense* leaves. Many of the populations from which we sampled were not bearing fruit at the time or the fruit were low in abundance compared to leaves so they were left undisturbed for conservation purposes. Clearly, further investigation is needed to determine the essential oil yield of other plant parts for the species.

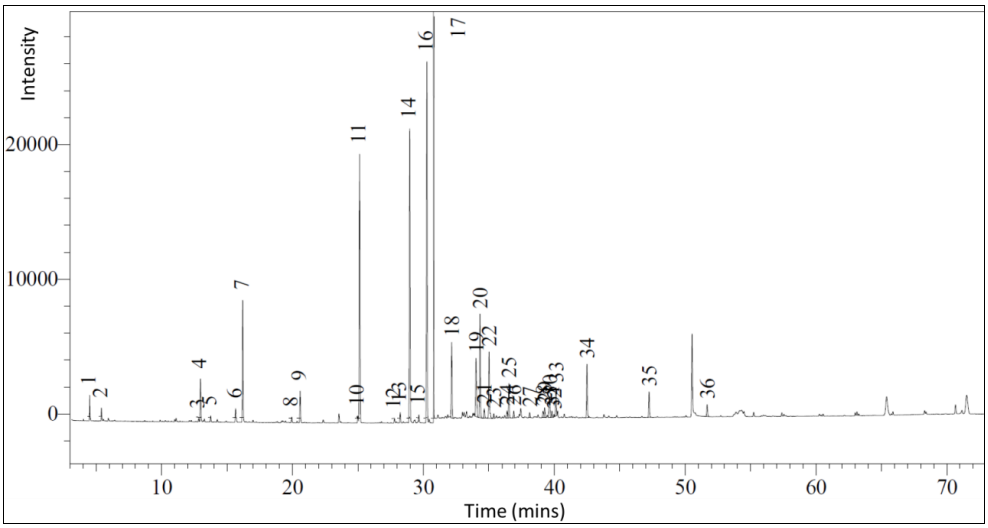

Fig 2: Example MS chromatogram for the *Illicium* project. This particular sample was the sesquiterpene-rich CACC, from the Cordillera Central.

3.2 Essential oil composition

Essential oil composition results are shown in Table B and an example chromatogram is shown in Figure 2. For all samples in aggregate, we identified 108 different compounds, 103 compounds in *I. ekmanii* and 34 from the oil from *I. hottense.* The identified compounds were grouped, at the bottom of Table B, according to six classifications: monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS), phenylpropanoids (P), and others (O). The total percentage of compounds identified ranged from 96.4 to 99.8%. Aside from the n-octane and n-eicosane internal standards, ten compounds were found across all samples, including linalool (3.22-17.15%), α-terpineol (0.41-1.99%), α-cubebene (0.19-0.65%), α-copaene (4.03-26.99%), β-elemene (0.26-0.93%), caryophyllene (5.43-35.70%), α-humulene (0.86-3.53%), δ-cadinol (0.2-0.79%), and τ-muurolol (0.92-4.33%). Interestingly, safrole was only found in two *I. ekmanii* samples, where it made up greater than ten percent of the oil in CACC (10.24%) and CSSAL (14.57%), but was

absent from *I. hottense*. Safrole was a component we expected to find based on previous *Illicium* reports, especially that of *I. florandium*, where it is the chief component at nearly 70% of the composition [26]. (E)-anethole, the signature compound from Old World *I. verum* due to commercialization, was detected only in a low amount in *I. hottense* (1.07%), providing more evidence in terms of volatile oils, for the divergence of this species [2] and the possible influence of Old World characteristics.

Table 2: Essential oil composition and oil yields for *I. ekmanii* from five sampling sites, and *I hottense* from Les-Cayes, Haiti. The essential oils were grouped according to six compound classifications at the bottom of the table. Results shown are from triplicate extractions for CCLM, CSSAL, and HOT and triplicate analytical replicates for CCCA, CCFJA, and CSIT.

RI-Calc	RI- LIT	Compounds	Class	*I. ekmanii*					*I. hottense*
				CC			CS		*L-C*
				LM	FJA	CA	SAL	IT	*GB*
800		Octane (IS)	IS						
831	835	Furfural	O	-	-	0.4	< 0.1	0.6	0.1
919	923	Tricyclene	MH	-	0.1	-	-	-	-
927	928	α-Thujene	MH	-	-	-	-	-	-
935	936	α-Pinene	MH	0.2 ± 0.1	0.3	-	< 0.1	-	-
950	950	Camphene	MH	0.4 ± 0.2	0.6	-	-	-	-
966	967	Furfural, 5-methyl-	O	0.2 ± 0.1	-	-	-	0.1	-
993	989	Myrcene	MH	-	-	-	< 0.1	0.1	-
1030	1030	Limonene	MH	0.2 ± 0.1	0.3	0.1	0.1	0.2	-
1032	1032	1,8-Cineole (Eucalyptol)	P	1.9 ± 1.0	0.8	1.5	0.4	-	-
1050	1047	β-Ocimene, (E)-	MH	-	-	0.2	-	0.2	-
1060	1060	γ-Terpinene	MH	-	-	-	0.0	-	-
1075	1075	Linalool oxide, (furanoid), cis-	O	-	-	-	-	0.1	0.2
1090	1087	Terpinolene	MH	-	0.7	0.6	0.6	1.0	-
1101	1099	Linalool	OM	6.7 ± 3.0	4.4 ± 0.2	4.5	3.2 ± 0.1	9.4 ± 0.3	17.2 ± 1.3
1106	1107	Hotrienol	OM	0.2 ± 0.1	-	-	< 0.1	-	0.9
1120		NI	NI	-	-	-	0.1	-	0.2
1123	1123	p-Menth-2-en-1-ol, cis	OM	-	-	-	-	-	-
1142	1137	p-Menth-2-en-1-ol, trans	OM	-	-	-	-	-	-
1165	1165	δ-Terpineol	OM	-	-	-	-	-	-
1180	1177	Terpinen-4-ol	OM	-	0.2	0.2	0.1	-	-
1188	1184	p-Cymenol-8-ol	OM	-	0.3	-	-	-	-
1193	1190	α-Terpineol	OM	0.7 ± 0.4	0.7	1.3	0.4 ± 0.1	2.3 ± 0.1	2.0 ± 0.3
1231	1229	Nerol	OM	-	-	-	0.1	0.2	0.5
1257	1255	Geraniol	OM	-	0.2	-	0.2	0.7	1.7 ± 0.2
1289	1284	Bornyl acetate	OM	8.9 ± 2.8	9.04± 0.3	0.3	0.3	-	-
1290	1285	Anethole, (E)-	P	-	-	-	-	-	1.1 ± 0.1
1292	1287	Safrole	P	-	-	10.2	14.6± 1.4	-	-
1340	1337	δ-elemene	SH	0.3 ± 0.1	-	-	-	-	-
1352	1351	α-Cubebene	SH	0.6 ± 0.1	0.7	0.2	0.4	0.2	0.3
1361	1358	Eugenol	P	-	-	0.4	0.6	0.2	-
1375	1370	α-Ylangene	SH	-	-	-	0.0	0.1	0.2
1379	1376	α-Copaene	SH	16.5 ± 2.2	27.0 ± 0.3	11.4	9.7 ± 0.9	19.3± 0.2	4.0 ± 0.8
1383		NI	NI	-	-	-	-	1.0	-
1385		NI	NI	0.5 ± 0.1	-	-	0.4	-	-
1387	1380	Geranyl acetate	O	-	-	-	-	-	0.6
1388	1384	β- Bourbonene	SH	0.3 ± 0.1	0.7	-	0.3	-	-
1389	1385	α-Funebrene	SH	-	-	-	-	0.1	-
1391	1387	β-Cubebene	SH	0.3 ± 0.1	-	-	-	-	-
1395	1390	β-Elemene	SH	0.9 ± 0.1	0.6	0.3	0.3	0.6	0.5
1400		NI	SH	-	-	-	0.1	0.3	-
1410	1402	Methyl eugenol	P	-	0.7	13.7	40.7 ± 2.8	-	2.3 ± 0.1
1412		NI	NI	0.2 ± 0.1	-	-	-	-	-
1414	1409	α-Gurjunene	SH	-	0.4 ± 0.1	-	-	-	-
1417	1407	α-Barbatene	SH	1.6 ± 0.2	-	-	0.9 ± 0.1	3.1 ± 0.1	-
1424	1420	Caryophyllene, (E)-	SH	15.2 ± 1.4	22.5 ± 0.1	32.4	5.4 ± 0.4	12.9 ± 0.1	35.7 ± 2.3
1433	1433	β- Copaene	SH	0.4 ± 0.1	0.2	-	0.1	0.1	0.4
1436	1432	Thujopsene, cis-	SH	0.4 ± 0.0	-	-	0.3	0.5	-
1441	1436	γ-Elemene	SH	0.8 ± 0.0	-	-	0.6	1.3	-
1444		NI	NI	-	-	-	0.0	0.3	-
1448	1440	β-Barbatene	SH	0.5 ± 0.1	-	-	0.1	0.5	-
1451	1452	Prezizaene	SH	0.4 ± 0.0	-	-	0.3	0.6	-
1455	1457	Cadina-3,5-diene	SH	0.6 ± 0.0	-	-	-	1.0 ± 0.1	-
1457	1442	Guaia-6,9-diene	SH	-	-	-	0.3	-	-
1459	1453	α-Humulene	SH	2.2 ± 0.1	2.6	3.1	0.9	2.0 ± 0.1	3.5 ± 0.2
1464	1451	Methyl isoeugenol, trans	P	-	0.3	-	-	-	-
1466		NI	SH	-	0.1	-	-	0.2	-

1478		NI	NI	-	0.4	-	-	-	-
1478	1475	Cadina-1(6),4-diene	SH	-	-	-	0.2	3.4 ± 0.1	-
1481	1476	γ-Muurolene	SH	0.9 ± 0.2	0.5	-	-	-	-
1483	1478	β-Chamigrene	SH	1.2 ± 0.3	-	-	0.6	2.0 ± 0.2	-
1485	1482	α-Amorphene	SH	5.7 ± 1.7	-	-	0.5 ± 0.1	0.4 ± 0.1	1.3 ± 0.1
1486	1481	Germacrene D	SH	-	0.7	-	-	-	-
1486		NI	NI	-	-	-	-	-	0.4
1491	1486	β-Selinene	SH	-	-	-	-	0.2	-
1492	1489	β-Guaiene, cis	SH	-	-	-	-	0.1	1.6
1496	1484	Muurola-4(15),5-diene, trans	SH	0.2 ± 0.0	-	-	-	0.2	-
1500	1502	Guaia-1(10),11-diene	SH	0.7 ± 0.1	0.3	-	-	1.4	-
1500	1493	α-Selinene	SH	-	-	-	-	-	2.1 ± 0.2
1501	1494	Bicyclogermacrene	SH	1.1 ± 0.1	-	-	-	-	-
1503	1491	Methyl isoeugenol, cis	P	-	2.8	3.0	0.3	-	12.1 ± 0.6
1506	1517	Myristicin	P	-	-	-	2.6 ± 0.2	-	-
1506	1498	α-Muurolene	SH	1.7 ± 0.1	-	-	1.9 ± 0.1	2.3	-
1512	1507	Cuparene	SH	2.4 ± 0.2	-	-	1.1 ± 0.1	3.6 ± 0.1	-
1513	1504	α-Farnesene	SH	-	0.5	3.9	-	-	-
1519	1511	δ-Amorphene	SH	-	1.3	-	0.6	-	-
1520	1523	Selina-3,7(11)-diene	SH	-	-	-	-	1.6	-
1521	1513	γ-Cadinene	SH	-	-	0.4	-	-	-
1523		NI	NI	1.4 ± 0.1	-	-	0.1	0.1	0.6
1529	1523	Calamenene, cis	SH	-	-	-	-	10.9±0.3	-
1529	1523	δ-Cadinene	SH	7.0 ± 0.5	4.9 ± 0.1	-	2.8 ± 0.3	-	2.5 ± 0.4
1531	1531	Cadina-1(2),4-diene, cis	SH		-	2.8	-	-	-
1535	1530	Epizonarene	SH	1.1 ± 0.2	-	-	0.4	0.7	-
1538		NI	NI		0.6	0.2	1.7	1.8	-
1539	1542	δ-Cuprenene	SH	2.2 ± 0.2	-	-	-	-	-
1543	1533	α-Cadinene	SH	0.2	0.3	-	0.1	0.2	-
1547	1540	α-Bisabolene, (E)-	SH	-	-	-	0.4	0.1	-
1549	1540	α-Calacorene	SH	-	0.4	-	-	0.3	0.2
1555	1548	Elemol	OS	-	-	-	-	0.2	-
1559		NI	NI	-	0.4	-	-	-	0.2
1565	1554	Elemicin	P	-	-	0.3	0.1	-	-
1569	1561	Nerolidol, (E)-	OS	-	-	1.4	0.3	0.5	0.8
1579	1570	Caryophyllene alcohol	OS	-	-	0.3	-	0.2	0.2
1584	1576	Spathulenol	OS	-	0.4	-	0.1	0.3	-
1590	1582	Globulol	OS	-	2.5 ± 0.2	0.2 ± 0.1	0.2	0.9	-
1592	1583	Gleenol	OS	0.8	-	-	-	-	1.0 ± 0.2
1598		NI	NI	-	0.2	-	-	0.1	-
1601	1595	Cubeban-11-ol	OS	-	-	-	-	0.3	-
1609	1588	Epiglobulol	OS	-	-	0.3	-	0.8	-
1611		NI	NI	-	-	-	-	-	-
1617	1608	Humulene epoxide II	OS	-	0.4	-	-	-	-
1622	1612	Cubenol, 1,10-di-epi-	OS	0.2	0.4	0.4	-	-	-
1625		NI	NI	-	-	-	-	0.1	-
1626	1626	1-Cubenol, epi-	OS	-	0.1	-	-	-	-
1630		NI	NI	-	-	-	-	0.1	-
1637	1630	Epicubenol	OS	1.0	-	-	-	0.1	0.3
1639	1631	γ-Eudesmol	OS	-	0.4	-	0.1	0.6	0.9
1641	1637	Cubenol	OS	0.8 ± 0.1	0.7	-	0.4	0.4	-
1644	1640	Caryophylla-4(12),8(13)-dien-5-α-ol	OS	-	0.5	-	-	-	0.4
1648	1638	Cadinol <epi-α->	OS	-	2.5	-	0.4	-	-
1649	1652	α-Cadinol	OS	3.5 ± 0.3	-	0.8 ± 0.4	1.3	2.1	0.5 ± 0.2
1654	1639	δ-Cadinol	OS	0.8 ± 0.1	0.6	0.2	0.4	0.6	0.3 ± 0.1
1658	1654	Eudesm-4-en-11-ol	OS	-	0.1	0.7 ± 0.3	-	-	-
1658	1650	β-Eudesmol	OS	-	-	-	-	0.2	0.4 ± 0.1
1662	1666	τ-Muurolol	OS	4.3 ± 0.7	2.5	1.1	1.5	3.6	0.9 ± 0.4
1665		NI	NI	-	1.0	-	-		0.9 ± 0.2
1679		NI	NI	-	0.6	-	-	0.1	-
1680	1688	Eudesma-4(15),7-dien-1-β -ol	OS						0.6 ± 0.1
1683	1682	Cadalene	SH	-	-	-	-		-
1687	1676	Mustakone	O	-	0.6	-	-		-
1728	1722	Farnesol, (2Z,6E)-	OS	-	-	2.2	-		-
1813	1801	Cadin-1,3,5-trien-5-ol	OS	-	-	-	-	0.2	-
1867	1854	Benzonic acid, 2-phenylethyl ester	O	-	-	1.1	-	-	-
1920	1906	Isopimara-9(11),15-diene	O	0.2	0.5	-	-	-	-
1972	1968	Sandaracopimaradiene	O	2.0 ± 0.4	-	-	0.7	0.2	-
1974	1978	Geranyl benzoate	O	-	-	-	-	-	0.6 ± 0.1
2000	2000	Eicosane (IS)	IS						

Number Compounds	48	54	34	59	70	40
Essential Oil Yield (% w/w dry leaf)	0.1 ± 0.0	0.6	0.6	1.1 ± 0.5	0.3	0.9 ± 0.2
Monoterpene Hydrocarbons (MH,5)	0.8	1.9	0.9	0.8	1.5	0.0
Others (O,5)	2.3	1.1	1.4	0.7	1.1	1.5
Oxygenated Monoterpenes (OM,5)	16.4	14.8	6.3	4.2	12.7	22.2
Oxygenated Sesquiterpenes (OS,5)	11.4	11.0	7.5	4.5	10.7	6.2
Phenylpropanoids (P,5)	1.9	4.6	29.3	59.2	0.2	15.4
Sesquiterpene Hydrocarbons (SH,5)	65.2	63.5	54.4	28.2	70.2	52.3
Total Identified (%)	98.0	96.8	99.8	97.7	96.4	97.6

The oils extracted from CCLM, CCFJA, CCCA, CSIT and HOT were all sesquiterpene-rich, ranging from 54.39% to 70.25% sesquiterpenes. The composition was strongly driven by contributions from α-copaene, caryophyllene, and δ-cadinene (Table B). At CSSAL, plant samples showed a phenylpropene-rich oil, where less than 28.19% were sesquiterpenes. This was driven by a large abundance of methyleugenol (40.71%) and safrole (14.57%), and a smaller contribution from a phenylpropanoid compound unique to the samples found on Hispaniola, myristicin (2.56%). Both phenylpropenes, safrole and myristicin, have been previously reported in *Illicium* and are psychoactive compounds that have been shown to have a variety of therapeutic uses [34] and toxicological implications [28,35].

The volatile oils in the leaves of *I. hottense* were dominated by β-caryophyllene (35.7 ± 0.3%), linalool (17.2 ± 0.3%), and cis-methyl isoeugenol (12.1 ± 0.2%) plus contributions from 37 other compounds. The oils were sesquiterpene-rich (52.30%) and had the most oxygenated monoterpenes (22.19%) of any sample. The sample extract also expressed phenylpropenes (15.42%), the bulk of which was cis-methyl eugenol (12.10%) but also included some (E)-anethole (1.15%). *I. floridanum* was shown to have oils dominated in monoterpenes and oxygenated monoterpenes [26], so our results suggest the volatile composition of *I. hottenses* to be most similar to *I. floridanum* in terms of categorical composition in New World species. The species appears to fall into the previously described sesquiterpene-rich chemotypical grouping [15].

4. Conclusion

We believe this study to be the first detailed analyses of foliar essential oil composition for *I. ekmanii* and *I. hottense.* Cordillera Septentrional samples of *I. ekmanii* showed more diversity in their essential oils based on the aggregate number of compounds detected, where an abundance of phenylpropenes (methyl eugenol and safrole) were observed in CSSAL, while CSIT showed little phenylpropanoid production and an abundance of sesquiterpenes. *I. hottense* leaf oil was made up of sesquiterpenes (β-caryophyllene and α-copaene) and oxygenated monoterpenes (linalool) and showed a similar composition as most *I. ekmanii* samples with the exception of the presence of (E)-anethole. More research is needed at the Septentrional sites, where *I. ekmanii* requires additional sampling and taxonomic review to better understand samples expressing more phenylpropenes compared to the sesquiterpene characteristics of Cordillera Central populations. If plant material is available, antimicrobial, and pharmaceutical activity testing would also be prudent.

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6. References

1. Oh I-C, Denk T, Friis EM. Evolution of *Illicium* (Illiciaceae): Mapping morphological characters on the molecular tree. Plant Syst Evol 2003;240:175-209.
2. Morris AB, Bell CD, Clayton JW, Judd WS, Soltis DE, Soltis PS. Phylogeny and divergence time estimation in *Illicium* with implications for New World biogeography. Syst Bot 2007;32(2):236-249.
3. Liu Y-N, Su X-H, Huo C-H, Zhang X-P, Shi Q-W, Gu Y-C. Chemical constituents of plants from the genus *Illicium*. Chem Biodivers 2009;6(7):963-989.
4. Padmashree A, Roopa N, Semwal AD, Sharma GK, Agathian G, Bawa AS. Star-anise (*Illicium verum*) and black caraway (*Carum nigrum*) as natural antioxidants. Food Chem 2007;104(1):59-66.
5. Najar B, Shortrede JE, Pistelli L, Buhagiar J. Chemical composition and *in vitro* cytotoxic screening of sixteen commercial essential oils on five cancer cell lines. Chem Biodivers 2020;17(1):e1900478.
6. Singh S, Verma R. Comprehensive review on pharmacological potential of *Illicium verum*, Chinese herb. Pharm Res Mod Chin Med 2024;10:100411.
7. Zou Q, Huang Y, Zhang W, Lu C, Yuan J. A comprehensive review of the pharmacology, chemistry, traditional uses and quality control of star anise (*Illicium verum* Hook. F.): An aromatic medicinal plant. Molecules 2023;28(21):7378.
8. Zhang X, Meng X, Wu J, Huang L, Chen S. Global ecological regionalization of 15 *Illicium* species: nature sources of shikimic acid. Chin Med 2018;13:31.
9. Li F, Yan T-T, Fu Y-Y, Zhang N-L, Wang L, Zhang Y-B, *et al*. New phenylpropanoid glycosides from *Illicium majus* and their radical scavenging activities. Chem Biodivers 2021;18(4):e2001012.
10. Kačániová M, Vukovic NL, Čmiková N, Bianchi A, Garzoli S, Ben Saad R, *et al*. Biological activity and phytochemical characteristics of star anise (*Illicium verum*) essential oil and its anti-salmonella activity on sous vide pumpkin model. Foods 2024;13(10):1505.
11. Lei J-D, Zhang S-B, Ding W-Z, Lv Y-Y, Zhai H-C, Wei S, *et al*. Antifungal effects of trans-anethole, the main constituent of *Illicium verum* fruit volatiles, on *Aspergillus flavus* in stored wheat. Food Control 2023;149:109721.
12. Huang Y, Zhao J, Zhou L, Wang J, Gong Y, Chen X, *et al*. Antifungal activity of the essential oil of *Illicium verum* fruit and its main component trans-anethole. Molecules 2010;15(11):7558-7569.

13. Wang G-W, Hu W-T, Huang B-K, Qin L-P. *Illicium verum*: A review on its botany, traditional use, chemistry and pharmacology. J Ethnopharmacol 2011;136(1):10-20.
14. Seigler DS. Phenylpropanoids. In: Plant Secondary Metabolism. Boston (MA): Springer US; 1998. p. 106-129.
15. Qinh NB, Dai DN, Than BV, Dung VT, Hang VT, Ogunwande IA. Volatile constituents of three *Illicium* plants. Rec Nat Prod 2016;10:806-811.
16. Wong Y, Lee P, Nurdiyana W. Extraction and antioxidative activity of essential oil from star anise (*Illicium verum*). Orient J Chem 2014;30:1159-1171.
17. Dũng NX, Chính ND, Leclercq PA. Volatile constituents of the fruit oil of *Illicium griffithii* Hook. f. et Thorns. from Vietnam. J Essent Oil Res 1995;7:451-452.
18. Dutta SC, Saha BN, Pathak MG, Kanjilal PB, Mathur RK. Essential oil of *Illicium griffithii* Hook. f. & Thorns. J Essent Oil Res 1997;9:227-228.
19. Chu SS, Liu SL, Jiang GH, Liu ZL. Composition and toxicity of essential oil of *Illicium simonsii* Maxim (*Illiciaceae*) fruit against the maize weevils. Rec Nat Prod 2010;4:205-210.
20. Howes M-JR, Kite GC, Simmonds MSJ. Distinguishing Chinese star anise from Japanese star anise using thermal desorption−gas chromatography−mass spectrometry. J Agric Food Chem 2009;57:5783-5789.
21. Guerrero Á, Ruiz-Vargas N. Biogeography of the *Cymbostemon* section (*Illicium*, *Schisandraceae*) in the New World. Sci Env Clim 2022;1:61-69.
22. Guerrero A. A revision of the species of *Illicium* subsection *Parviflora* (*Illiciaceae*). Gainesville (FL): University of Florida; 1997.
23. Guerrero A, Judd WS, Morris AB. A new species of *Illicium* subsection *Parviflora* (*Illiciaceae*) from the Massif de la Hotte, Haiti. Brittonia 2004;56:346-352.
24. Garcia RG, Peguero JF, Veloz A, Clase T. Red List of the vascular flora in the Dominican Republic. 2016.
25. Timyan J. IUCN Red List of Threatened Species: *Illicium ekmanii*. IUCN Red List of Threatened Species 2022.
26. Tucker AO, Maciarello MJ. Volatile oils of *Illicium floridanum* and *I. parviflorum* (*Illiciaceae*) of the southeastern United States and their potential economic utilization. Econ Bot 1999;53:435-438.
27. Barman R, Kumar Bora P, Saikia J, Konwar P, Sarkar A, Kemprai P, *et al*. Hypothetical biosynthetic pathways of pharmaceutically potential hallucinogenic metabolites in *Myristicaceae*, mechanistic convergence and co-evolutionary trends in plants and humans. Phytochem 2024;218:113928.
28. Gad SC. Safrole. In: Wexler P, editor. Encyclopedia of Toxicology. 4th ed. Oxford: Academic Press; 2024. p. 407-412.
29. Kemprai P, Protim Mahanta B, Sut D, Barman R, Banik D, Lal M, *et al*. Review on safrole: identity shift of the 'candy shop' aroma to a carcinogen and deforester. Flavour Frag J 2020;35(1):5-23.
30. Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. 4.1 ed. Carol Stream (IL): Allured Pub. Corp.; 2017.
31. Babushok VI, Linstrom PJ, Zenkevich IG. Retention indices for frequently reported compounds of plant essential oils. J Phys Chem Ref Data 2011;40(4):043101.
32. National Institutes of Standards and Technology. NIST Mass Spectral Library 2014. Gaithersburg (MD).
33. Yang E-C, Hsieh Y-Y, Chuang L-Y. Comparison of the phytochemical composition and antibacterial activities of the various extracts from leaves and twigs of *Illicium verum*. Molecules 2021;26(13):3909.
34. Seneme EF, dos Santos DC, Silva EMR, Franco YEM, Longato GB. Pharmacological and therapeutic potential of myristicin: a literature review. Molecules 2021;26(19):5914.
35. Hallström H, Thuvander A. Toxicological evaluation of myristicin. Nat Tox 1997;5:186-192.