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Integrated Analysis of Vietnamese *Illigera trifoliata* ssp. *cucullata* (Merr.) Kubitzki), Leaf and Stem Essential Oils by GC-FID/GC-MS and ^{13}C NMR

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Abstract: *Illigera trifoliata* ssp. *cucullata* (Merr.) Kubitzki (Hernandiaceae) is a liana distributed mainly in the tropical regions of Asia. Nothing is known on the phytochemicals produced by this species. Oil samples have been isolated from leaves, and stems, and analyzed by combination of chromatographic and spectroscopic techniques [GC(FID), GC-MS and ^{13}C NMR]. The compositions of leaf and stem oils were dominated by α-phellandrene (25.8 and 29.2%), α-pinene (26.0 and 9.5%) and β-phellandrene (12.8 and 15.8%), followed by limonene (5.5 and 8.1%) and β-pinene (7.5 and 3.0%). The stem essential oil from *Illigera trifoliata* ssp. *cucullata* exhibited antibacterial activity against *Bacillus subtilis* and *Escherichia coli* with MIC values of 4000 µg/mL and IC_{50} values of 1824,56 ±136 and 2666,66 ±257 µg/mL, respectively.

Keywords: *Illigera trifoliata* ssp. *cucullata;* essential oil composition; monoterpene hydrocarbons-rich oil**;** α-phellandrene; β-phellandrene; α-pinene.

1. Plant Source

I. trifoliata ssp. *cucullata* is a liana with brown and glabrous stem, 3-foliolate leaves, purple-green or green flowers, two-winged fruit with suborbicular or ligulate wings, 4-4.5 cm wide [1]. It grows wild on margins of evergreen forest, forest edges in open sunny places, in ravines, at altitude 100-1000 m. It is distributed in Andaman Islands, Laos, Thailand, Sumatra, and Peninsular Malaysia as well as in Vietnam [2].

The plants of *I. trifoliata* ssp. *cucullata* were collected in Pa Co-Hang Nature Reserve, Pa Co Commune, Moc Chau district, Hoa Binh province, located in North-Western Vietnam in October 20, 2020. Plant material was authenticated by Dr Do Van Hai (plant taxonomist, IEBR). A voucher specimen (N° TN-50) was deposited at the Herbarium of Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (HN).

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Illigera trifoliata ssp. *cucullata* (Merr.) Kubitsky leaf and stem essential oils

2. Previous Studies

Some phytochemical studies on chemical composition, structure, and bioactivity of compounds isolated from some species of genus *Illigera* have been presented in the literature [3-10]. To the best of our knowledge, nothing has been reported on phytochemicals produced by *Illigera trifoliata* whatever the sub-species.

3. Present Study

In continuation of our on-going work on the characterization of Vietnamese medicinal and aromatic plants through the composition of their essential oils [11,12], we report for the first time on the compositions of *Illigera trifoliata* ssp. *cucullata* (Merr.) Kubitzki (Hernandiaceae) leaf and stem essential oils.

The genus *Illigera* consists of *ca*. 20 species and is characterized by their invariably winged fruits, the wings of which are developed from two or more angles of the ovary. *Illigera trifoliata* (Griff.) Dunn (Hernandiaceae), also known as *Coryzadenia trifoliata* Griff. or *Illigera kurzii* C.B. Clarke, contains three infraspecific taxa, including *I. trifoliata* ssp. *cucullata* (Merr.) Kubitzki [13]. Some species of *Illigera* are used in traditional Vietnamese medicine to treat rheumatism, bone pain, febrile seizures, bright-yellow urine, hemoptysis, scabies and boils [14, 15].

Leaves and stems of *Illigera trifoliata* ssp. *cucullata* were hydrodistilled separately (4-5h) using a Clevenger-type apparatus. Yields, calculated from dry material were 0.35% and 0.07% (v/w), respectively for leaves and stems. Both oil samples were submitted to GC(RI), GC-MS and ^{13}C NMR analyses, following a method developed at the University of Corsica [16,17]. In total, 58 components have been identified. They accounted for 99.4% (leaf) and 97.2% (stem) of the whole composition, respectively.

Table 1. The Composition of *Illigera trifoliata* ssp. *cucullata* leaf and stem essential oils

	Compound[a]	Ria[litt]	Rip[litt]	RIa	RI p	%L	%S	Identification mode
1	α-Thujene	926	1027	924	1022	0.4	0.4	RI, MS
2	α-Pinene	934	1025	932	1022	26.0	9.5	RI, MS, ^{13}C NMR
3	Camphene	947	1068	945	1066	2.0	1.5	RI, MS, ^{13}C NMR
4	Thuja-2,4(10)-diene	945	1122	948	1128	0.1	t	RI, MS
5	Oct-1-en-3-ol	966	1444	963	1449	t	0.1	RI, MS
6	Sabinene	968	1122	967	1123	0.5	0.2	RI, MS, ^{13}C NMR
7	β-Pinene	973	1110	972	1112	7.5	3.0	RI, MS, ^{13}C NMR
8	Myrcene	983	1161	982	1162	4.4	3.7	RI, MS, ^{13}C NMR
9	α-Phellandrene	999	1168	1000	1168	25.8	29.2	RI, MS, ^{13}C NMR
10	δ-3-Carene	1007	1147	1007	1149	0.1	0.2	RI, MS
11	*p*-Cymene	1015	1270	1013	1273	1.8	5.7	RI, MS, ^{13}C NMR
12	Limonene*	1024	1198	1023	1203	*5.5**	*8.1**	RI, MS, ^{13}C NMR
13	β-Phellandrene*	1021	1209	1023	1212	*12.8**	*15.8**	RI, MS, ^{13}C NMR
14	(*Z*)-β-Ocimene	1029	1234	1026	1233	0.1	0.1	RI, MS
15	(*E*)-β-Ocimene	1038	1250	1038	1250	2.9	3.2	RI, MS, ^{13}C NMR
16	γ-Terpinene	1050	1245	1050	1246	0.1	0.1	RI, MS
17	Terpinolene	1079	1282	1080	1283	0.1	0.1	RI, MS
18	Linalool	1086	1543	1085	1547	0.3	0.4	RI, MS, ^{13}C NMR
19	3-Acetoxyoct-1-ene	1091	1380	1095	1378	0.3	0.4	RI, MS, ^{13}C NMR
20	*cis*- *p*-Menth-2-en-1-ol	1108L	1559L	1110	1563	t	0.2	RI, MS
21	*trans*-*p*-Menth-2-en-1-ol	1124L	1624L	1126	1616	0.2	0.1	RI, MS

Table 1 continued..

22	*trans*-Verbenol	1134	1680	1131	1678	0.7	0.2	RI, MS, ^{13}C NMR
23	*p*-Cymen-8-ol	1165	1848	1162	1848	0.0	0.1	RI, MS
24	Terpinen-4-ol	1164	1601	1164	1602	0.1	0.2	RI, MS
25	α-Terpineol	1176	1694	1175	1696	0.1	0.2	RI, MS
26	Estragole	1178	1671	1182	1671	t	0.1	RI, MS
27	Neral	1220	1678	1217	1682	0.3	0.4	RI, MS, ^{13}C NMR
28	Geraniol	1239	1839	1237	1845	0.1	0.2	RI, MS
29	Geranial	1247	1725	1245	1733	0.3	0.6	RI, MS, ^{13}C NMR
30	Carvacrol	1283	2211	1278	2195	0	0.1	RI, MS
31	δ-Elemene	1340	1469	1337	1468	0.1	0.1	RI, MS
32	α-Copaene	1375	1491	1377	1489	0.4	1.1	RI, MS, ^{13}C NMR
33	β-Elemene	1388	1591	1389	1587	0.3	0.3	RI, MS
34	(*E*)-β-Caryophyllene	1419	1598	1419	1594	1.8	1.4	RI, MS, ^{13}C NMR
35	*trans*-α-Bergamotene	1431	1576	1434	1581	t	0.1	RI, MS
36	α-Humulene	1449	1667	1452	1666	0.3	0.3	RI, MS
37	Germacrene D	1476	1708	1477	1705	0.4	0.5	RI, MS, ^{13}C NMR
38	β-Selinene	1481	1717	1483	1714	0.1	0.1	RI, MS
39	Bicyclogermacrene	1490	1734	1493	1729	1.8	0.9	RI, MS, ^{13}C NMR
40	(*E*,*E*)-α-Farnesene	1496	1744	1497	1745	0.2	0.1	RI, MS
41	β-Bisabolene	1500	1727	1502	1722	0.1	0.8	RI, MS, ^{13}C NMR
42	Calamenene#	1510	1823	1509	1828	0	0.1	RI, MS
43	δ-Cadinene	1514	1756	1511	1752	0	0.2	RI, MS
44	β-Elemol	1536	2079	1536	2076	0.4	0.5	RI, MS, ^{13}C NMR
45	*cis*-7-*epi*-Sesquisabinene hydrate	1543L	1991L	1543	1995	0.0	0.2	RI, MS
46	(*E*)-Nerolidol	1550	2036	1550	2034	0.2	0.5	RI, MS, ^{13}C NMR
47	Spathulenol	1566	2126	1565	2118	0.2	0.6	RI, MS, ^{13}C NMR
48	Caryophyllene oxide	1570	1986	1572	1978	0.1	0.1	RI, MS
49	Globulol	1579	2082	1577	2082	0	0.2	RI, MS
50	Zingiberenol I	1599L	2107L	1601	2107	0	0.2	RI, MS
51	10-*epi*-γ-Eudesmol	1608	2106	1623	2108	0	0.1	RI, MS
52	β-Eudesmol	1634	2238	1637	2223	0	1.3	RI, MS, ^{13}C NMR
53	Selin-11-en-4α-ol	1641	2252	1639	2246	0.5	1.6	RI, MS, ^{13}C NMR
54	α-Eudesmol	1641	2223	1642	2220	t	0.2	RI, MS
55	β-Bisabolol	1659	2143	1655	2148	0	0.2	RI, MS
56	*epi*-β-Bisabolol	1657	-	1657	-	0	0.2	RI, MS
57	α-Bisabolol	1668	2213	1668	2210	0	0.4	RI, MS, ^{13}C NMR
58	*epi*-α-Bisabolol	1674	2214	1671	2213	0	0.6	RI, MS, ^{13}C NMR
	Total					99.4	97.2	

[a] Order of elution and percentages are given on apolar column (BP-1), excepted those whose names are followed by an asterisk (*), percentages on polar column (BP-20) ; #correct isomer not identified; RI^{litt} ref [18] except components 20 and 21, ref [19], component 45, ref [20] and component 50, ref [17].

The two compositions were similar and they were characterized by the occurrence of monoterpene hydrocarbons. The compositions of leaf and stems oils were dominated by α-phellandrene (25.8 and 29.2%), α-pinene (26.0 and 9.5%) and β-phellandrene (12.8 and 15.8%),

followed by limonene (5.5 and 8.1%) and β-pinene (7.5 and 3.0%). Other monoterpene hydrocarbons present at appreciable contents were myrcene (4.4 and 3.7%), camphene (2.0 and 1.5%), p-cymene (1.8 and 7.7%), (E)-β-ocimene (2.9 and 3.2%). Oxygenated monoterpenes were identified at low content, each one accounted for less than 1% in both oil samples. Various sesquiterpene hydrocarbons were present at low content, except (E)-β-caryophyllene (1.8 and 1.4%) and bicyclogermacrene (1.8 and 0.9%). Oxygenated sesquiterpenes were a little bit more important in stem oil than in leaf oil, for instance, β-eudesmol 1.3% and selin-11-en-4α-ol 1.6%. Concerning compound 45, the MS library suggested sesquisabinene hydrate and *cis*-7-epi-sesquisabinene hydrate was selected by comparison with the retention indices with those of the four diastereoisomers identified in *Cedrelopsis grevei* essential oil [20].

The compositions of *I. trifoliata* ssp. *cucullata* leaf and stem oils resembled to that of *I. Jaromatic* S.Z. Huang et S.L. Mo, dominated by monoterpene hydrocarbons, α-pinene, β-pinene, α-phellandrene, p-pymene, β-phellandrene, beside α-bisabolene and α-eudesmol [3]. It differed drastically from that of *I. rhodantha* Hance, dominated by n-hexadecanoic acid, tridecanoic acid, beside linalool and (*E*)-β-caryophyllene [4].

The essential oil samples were then subjected to microbroth dilution assays [6-7] to determine the minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values using 7 strains of microorganisms: *Staphylococcus aureus*, *Bacillus subtilis*, and *Lactobacillus fermentum*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. The results of the assay obtained after 16-24 hours of incubation are presented in Table 2. The stem essential oil from *Illigera trifoliata* ssp. *cucullata* exhibited antibacterial activity against *Bacillus subtilis* and *Escherichia coli* with MIC values of 4000 μg/mL and IC_{50} values of 1824,56 ±136 and 2666,66 ±257 μg/mL, respectively. The oil from *Illigera trifoliata* ssp. *cucullata* leaves had no inhibitory effects on test microorganisms (Table 2). The antimicrobial activity of essential oils can be derived from their main compounds. α-pinene, β-pinene, α-phellandrene being main components of essential oil samples in the present study may contribute the role in antimicrobial and anti-cholinesterase activities because they belong to group of monoterpen ($C_{10}H_{16}$) as previously attributed [21].

Table 2. MIC and IC_{50} of essential oils from leaves and stems of *Illigera trifoliata* ssp. *cucullata*

Esential oil samples	***Illigera trifoliata* ssp. *cucullata* leaves**		***Illigera trifoliata* ssp. *cucullata* stems**	
Value (μg/mL)	IC_{50}	MIC	IC_{50}	MIC
S. aureus	-	-	-	-
B. subtilis	>8000	>8000	1824,56 ±136	4000
L. fermentum	>8000	>8000	>8000	>8000
S. enterica	>8000	>8000	>8000	>8000
E. coli	>8000	>8000	2666,66 ±257	4000
P. aeruginosa	-	-	-	-
C. albicans	-	-	-	-

In the previous study, a new aporphine alkaloid (illigerine B) extracted from *Illigera aromatic* exhibited moderate cytotoxic activity against the three tumor cell types Hela, SMMC7721, and Bcap37, with IC_{50} values of 12.40 ± 0.78, 32.61 ± 2.05, and 28.69 ± 1.80 μg/mL [18]. Five aporphines (actinodaphnine, N-methylactinodaphnine, launobine, dicentrine, O-methylbulbocapnine) and an oxoaporphine (liriodenine) isolated from the stems of *Illigera luzonensi*s showed significant antiplatelet aggregation and actinodaphnine and dicentrinone exhibited significant vasorelaxant activities, respectively [19]. Other research revealed that four monoterpenoids (illigerate A, C, F and G) isolated from *Illigera aromatica* had anti-inflammatory activity with IC_{50} values of 71.5±7.3, 74.7±5.6, 48.0±7.4 and 65.1±3.7μM, respectively [20]. Thus, the previous studies on the biological activity were only from extracts of the species, there were no studies on the antimicrobial activity of essential oils.

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Supporting Information

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