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Constituents of essential oils from *Illicium micranthum* Dunn and *Illicium griffithii* Hook. f. et Thoms

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Abstract
The chemical composition of volatiles from the fruit of *Illicium micranthun* Dunn and the root of *Illicium griffithii* Hook. f. et Thoms have been studied. The essential oils were obtained by hydrodistillation and analyzed by GC (FID) and GC-MS. The components were identified by MS libraries and their LRIs. The essential oil content was 0.12% (v/w) for *I. micranthun*, as well as 0.12% (v/w) and 0.15% (v/w), respectively *I. griffithii* from Pù Mát National Park, Nghệ An Province and Tam Đảo National Park, Vinh Phuc Province, calculated on a dry weight basis. (*E*)-Nerolidol (18.4%) and linalool (11.0%) are the quantitatively significant compounds of the oil of *I. micranthun*. The main compounds of Sa Pa *I. griffithii* oil sample were α-cadinol (24.1%), τ-muurolol (9.8%), α-curcumene (6.9%), aromadendrene (6.8%) and cuparene (6.7%) while borneol (18.8%) and 1,8-cineole (13.2%) occurred in higher quantity in Tam Đảo National Park *I. griffithii* oil sample. The chemical composition of *I. micranthun* fruit and *I. griffithii* root essential oils are being reported for the first time.

Keywords: *Illicium micranthun*, *Illicium griffithii*, Essential oil composition, Monoterpenes, Sesquiterpenes

1. Introduction
Illicium is a genus of flowering plants treated as part of the family Schisandraceae, or alternately as the sole genus of the Illiciaceae [1]. *Illicium micranthum* Dunn, Hooker's Icon is a tree or shrub about 10 m tall. The leaf in clusters of 3-5 are obvate and thinly leathery. The flowers are red to orange red in colour. Flowering takes place between April and June while fruiting occurs from July to September. Extract of the plant possessed antimicrobial potential [2]. The phytochemicals isolated from plant includes illicinolide C with antioxidant property [3], micranthumnins A-G which serves as an anti-acetylcholinesterase [4], (1*R*,2*S*)-1,2-epoxyneomajucin, (2*R*)-2-hydroxyneomajucin and (2*R*)-2-hydroxymajucin [5]. In addition, β-sitosterol, quercetin, rutin, quercetin-3-O-α-L-rhamnoside, magnolol, dictagymnin, (+)-syringaresinol, (+)-diaeudesmin and gallic acid were also isolated from the plant [6]. Other phytochemicals of *I. micranthum* include micranthumoside, 7-β-D-glucosyl pseudomajucin, 4, 7, 9-trihydroxy-3, 3′-dimethoxy-8-O-4′-neolignan-9-O-α-L-rhamnopyranoside, isolariciresinol -3α-O-β-D-glucopyranoside, rutin, myricetin-3-O-α-L-rhamnoside, kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, kaempferol-8-C-α-L-rhamnopyranosyl-(1→2) -β-D-glucopyranoside, icariside E3, and shikimic acid [7]. The ethyl acetate layer of methanol extract from the fruits of *I. micranthum* afforded gynurenol, β-elemol, *epi*-carrisone, 6-hydroxy-eudesm-4(14)-ene, β-eudesmol, *trans*-eudesmane-4, 11-diol, 1*R*, 2*R*, 4*R*-trihydroxy-*p*-menthane, 2-isopropenyl-5-methyl-cyclohexanol, isopulegol, 4-isopropenyl-1-methyl-cyclohexanol, palmitic acid, triacontanoic acid, *p*-hydroxy-benzoic acid and β-daucosterol [8].
The essential oil of the pericarps contained high contents of safrole, linalool and limonene [9]. The composition of the pericarps oil obtained from China [10] was dominated by limonene (17.9%) and β-caryophyllene (8.6%). 1, 8-Cineole (21.47%), α-pinene (10.40%) and limonene (8.09%) were the significant compounds of the seed oil of *I. micranthum* [11]. The chemical constituents of the leaf oil of *I. micranthum* [12] was characterized by the abundance of 1, 8-cineole (8.4%), linalool (7.7%), (*E*)-nerolidol (7.6%) and sabinene (7.1%).
Illicium griffithii Hook. f. et Thoms is a large shrub of 3-4.5 m height. The plant flowers during January to April and fruiting occur by the end of April [13]. Its fruit is composed of compressed, beaked, incurved carpels, each containing one seed arranged in a single whorl.

The fruits are used medicinally to treat cough, sinusitis, toothache, regurgitating, dyspepsia, abdominal pain, and food poisoning and are considered carminative, stomachic, and galactagogic. The antioxidant [14, 15], antimicrobial [16] of the extracts from the fruits of the plant have been documented. Extracts from the fruits of the plant possessed cytotoxicity, anti-diabetes and free radical scavenging activities [15]. The plant is known to contained shikimic acid [17, 18], p-menth-1(7), 4(8)-diene-3-O-B-D-glucoside [19]. Two phenolic compounds 3, 4-dihydroxybenzoic acid and 3, 4, 5-trihydroxybenzoic acid were isolated for the ethyl acetate extracts of the fruits *I. griffithii* and showed promising cytotoxic and antioxidant activities [15].

The main constituent of the essential oils were linalool [20] as well as 4-methyl-6-(2-propenyl)-1, 3-benzodioxole (22.64%) and linalool (12.05%) from Indian sample [21]. The essential oil of *I. griffithii* fruit from India [22] consists mainly of linalool (19.6%), p-methoxy phenyl acetone (11.8%), terpinen-4-ol (11.0%), limonene (10.6%) and safrole (10.1%). However, α-pinene (19.4%), linalool (19.0%), limonene (13.2%) and 1, 8-cineole (9.3%) were found to predominate in the fruit oil from Vietnam [23]. The composition of the oils of short peduncle harvested in Vietnam [24] were safrole (51.6–65.3%) while the root bark contained 4-methoxysafrole (19.6%). In addition, safrole and methyl eugenol were the main compounds in the leaves and stems oils [25]. The major compounds found in the hexane extract of the fruit of *I. griffithii* analyzed by GC/MS were myristicin (29.20%), linalool (10.70%) and germacrene-D (7.83%) while myristicin (25.45%), linalool (9.28%) and 1-hydroxy-2-(prop-2-enyl)-4, 5-methylenedioxybenzene (5.85%) were present in the ethyl acetate extract [16]. The volatile composition of samples from India and Malaysia [10] comprised of (*E*)-caryophyllene (14.9% and 17.3%) and methoxyeugenol (43.8% and 57.2%). The essential oil was found to be effective against *Staphylococcus aureus, Aspergillus niger, Penicillium* spp. and *Saccharomyces cerevisiae* [21]. The main objective of this study was to report the compounds identified in the volatile oils obtained by hydrodistillation of the fruits of *I. micranthum* and roots of *I. griffithii* collected from Vietnam. This is in continuation of an extensive research on the phytochemical analysis of Vietnamese grown plants [12].

2. Experimental

2.1 Plants collection

Mature fruits of *I. micranthum* were obtained from Pù Mát National park, Nghệ An Province, Vietnam, in September 2013. Roots of *I. griffithii* were collected from Pù Mát National park, Nghệ An Province and Tam Đảo National park, Vinh Phuc Province, Vietnam, in July 2011. The plant samples were identified by Dr. Dai DN. Voucher specimens NVH 40 (*I. micranthum*), NVH 19 (*I. griffithii*, Pù Mát) and BVT 20 (*I. griffithii*, Tam Đảo) were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Hydrodistillation of essential oils

About 0.5 kg of air-dried samples was shredded and the oil was obtained by separate hydrodistillation for 3 h at normal pressure, according to the Vietnamese Pharmacopoeia [26]. Oil samples were colourless.

2.3 Analysis of the oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm). Temperature parameters: column oven- 40 ºC, injection pot-250 ºC, detector-260 ºC. Time programming: 40 ºC for 2 min, temperature raise to 220 ºC (10 min hold) at 4 ºC/min. Carrier gas used was H_2 (1 mL/min), split ratio 10:1, volume injected: 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. Retention indices (RI) value of each component was determined relative to the retention times of a homologous *n*-alkane series with linear interpolation on the HP-5MS column. GC/MS was performed on HP 5973 MSD mass spectrometer with HP 6890N Plus GC system fitted with a fused silica capillary HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm). The conditions were the same as described above for GC with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

2.4 Identification of the constituents

Peaks were identified by comparison of relative GC retention indices with standards from literature, retention indices on HP-5 column, peak enrichment on co-injection with authentic standard wherever possible and comparison of mass spectra with literature data [27, 28].

3. Results and discussion

Table 1 indicates the chemical constituents present in the oil, their percentages as well as retention indices on HP-5MS column. The essential oil content of *I. micranthum* was 0.12% (v/w), calculated on a dry weight basis. Sesquiterpenes (41.2% hydrocarbons vs. 34.2% oxygenated) compound dominated in the essential oil of the fruit of *I. micranthum.* Monoterpenes occurred in lower quantity (2.2% hydrocarbons vs. 13.5% oxygenated). The chemical constituents of the fruit oil of *I. micranthum* was characterized by the abundance of (*E*)-nerolidol (18.4%), linalool (11.0%), α-cadinol (7.6%) and δ-cadinene (7.0%). The minor compounds present in the oil were β-caryophyllene (3.5%), τ-Muurolol (3.4%), α-copaene (3.1%), γ-gurjunene (3.0%) and α-amorphene (3.0%). Although no report could be seen on the fruit oil content of this plant, the chemical pattern was found to differ from those of the leaf, pericarps and seed oils. The main variations are the conspicuous lower contents of α-pinene, limonene, 1, 8-cineole and β-caryophyllene when compared with the leaf [12], pericarps [9, 10] and seed oils [11].

Sesquiterpene hydrocarbons (47.5%) and oxygenated counterparts (43.4%) constituted the bulk of Pù Mát *I. griffithii* oil sample. The main constituents identified in the oil were mainly sesquiterpene compounds namely α-cadinol (24.1%), τ-muurolol (9.8%), α-curcumene (6.9%), aromadendrene (6.8%) and cuparene (6.7%). Monoterpeme compounds are rarely common. The total content of monoterpene compounds was 3.7%. On the other hand, diverse chemical classes of compounds present in Tam Đảo oil *I. griffithii* oil were monoterpene hydrocarbons (15.2%), oxygen-containing monoterpene (52.0%) and sesquiterpene hydrocarbons (19.5%). However, the main monoterpene compounds comprised of borneol (18.8%), 1,8-cineole (13.2%), α-terpineol (8.1%) and linalool (6.6%). τ-Muurolol (6.1%) was the only sesquiterpene compound identified in significant amount. The present compositional pattern of the studied oil samples of *I. griffithii* were found to differed from previous studies on other parts of the plant. For example, 4-

methoxysafrole (from root), limonene (fruit), safrole (root bark, leaves and stems) and methyl eugenol (from leaves and stems) were conspicuously absent in the present oil samples. In addition, the quantity of α-pinene and linalool (19.0%) were lowered when compared with previous studies [23-25]. However, the present main terpene compounds such as borneol, α-cadinol, τ-muurolol, α-curcumene, aromadendrene and cuparene were not reported previously to be of significant quantity in previous analysis. The chemical compositions of essential oils from other *I. griffithii* plants from other part of the world have been reported. 4-Methyl-6-(2-propenyl)-1,3-benzodioxole [18], p-methoxy phenyl acetone, terpinen-4-ol and limonene [22] as well as and 4-ethyl-6-(2-propenyl)-1,3-benzodioxole[16], (*E*)-caryophyllene and methoxyeugenol [10] that are characteristics of India and Malaysia oil samples were not identified in this study.

In the same vein, the essential oils from other *Illicium* plants grown in other part of the world have been studied. trans-Anethole (89.5%) was the main compound of *I. verum* [29] while *I. fargesii* [30] contained α-terpineol (11.4%), carvone (10.9%), d-limonene (9.8%). However, safrole (54.09 %) and myristicin (22.24%) were present in *I. henryi* [31]. Linalool (22.53%) and linaly acetate (13.93%) were identified in *I. floridanum* while safrole (68.14%), linalool (13.18%) and methyl eugenol could be seen prominent in *I. parviflorum* [32]. When the results of the present analysis were compared with the constituents of other *Illicium* plants from Vietnam and elsewhere, it was found that each sample has its own composition pattern different from other. Several compounds that were present in other *Illicium* oils were not identified in the studied oils of *Illicium* plants.

Table 1: Constituents of essential oils of *I. micranthum* and *I. Griffithii*

Compounds[a]	RI (Cal.)	RI (Lit.)	Percentage (± SD) [b]		
			I. micranthum	*I. griffithii*[c]	*I. griffithii*[d]
Tricyclene	926	921	-	-	0.8
α-Pinene	939	932	0.5	1.0	2.8
Camphene	953	946	-	-	3.9
β-Pinene	980	976	0.4	-	0.1
β-Myrcene	990	987	-	-	0.2
δ-3-Carene	1011	1008	-	-	3.7
α-Terpinene	1017	1014	0.1	-	0.1
o-Cymene	1024	1022	-	-	3.5
p-Cymene	1026	1024	-	-	0.2
Limonene	1032	1030	0.3	-	-
1,8-Cineole	1034	1032	0.4	0.5	13.2
(*E*)-β-Ocimene	1052	1044	0.5	-	-
γ-Terpinene	1061	1056	0.2	-	0.1
Linalool oxide	1080	1080	-	-	0.3
α-Terpinolene	1090	1089	0.2	-	-
Linalool	1100	1095	11.0	0.1	6.6
Fenchyl alcohol	1122	1120	-	-	0.3
Camphor	1145	1141	-	0.5	0.3
Isoborneol	1160	1160	-	-	0.3
Borneol	1167	1167	-	0.8	18.8
Terpinen-4-ol	1177	1175	0.6	0.4	1.5
α-Terpineol	1189	1186	0.8	0.4	8.1
trans-Carveol	1217	1217	-	-	0.1
Methyl carvacrol	1245	1245	-	-	1.3
Geraniol	1253	1249	0.6	-	0.1
Bornyl acetate	1289	1287	-	-	1.0
2-Undecanone	1291	1293	0.2	-	-
Bicycloelemene	1327	1337	0.2	1.6	-
δ-Elemene	1340	1335	0.5	-	-
α-Copaene	1377	1374	3.1	0.5	1.8
Geranyl acetate	1381	1379	0.1	-	-
β-Cubebene	1388	1387	0.9	-	-
β-Elemene	1391	1389	1.4	-	-
α-Gurjunene	1412	1409	0.2	5.1	0.3
β-Caryophyllene	1419	1417	3.5	-	0.4
β-Cedrene	1421	1419	1.6	-	1.9
Widdrene	1431	1431	0.2	-	-
α-Thujopsene	1433	1429	2.2	-	-
trans-α-Bergamotene	1435	1431	1.0	-	-
Aromadendrene	1441	1439	1.0	6.8	2.1
α-Humulene	1454	1452	1.5	0.8	0.3
γ-Gurjunene	1477	1475	3.0	1.1	-
γ-Muurolene	1480	1478	1.0	3.0	-
α-Amorphene	1485	1484	3.0	1.2	1.1
β-Selinene	1486	1486	0.2	-	1.9
epi-Bicyclosesquiphellandrene	1489	1487	-	0.2	-
δ-Selinene	1493	1493	-	-	2.3
α-Selinene	1493	1494	-	1.0	0.8

Zingiberene	1494	1493	0.2	4.1	0.4
Epizonarene	1495	1495	-	-	0.1
Cadina-1,4-diene	1496	1496	-	2.6	-
Bicyclogermacrene	1500	1500	1.9	-	-
α-Muurolene	1500	1501	-	1.5	-
Cuparene	1500	1504	2.0	6.7	1.2
β-Bisabolene	1506	1504	-	-	0.3
(*E*,*E*)-α-Farnesene	1508	1505	1.2	-	-
δ-Cadinene	1525	1522	7.0	4.2	2.4
α-Curcumene	1536	1534	-	6.9	0.8
α-Calacorene	1546	1544	1.1	0.2	0.7
Elemol	1550	1548	0.4	-	-
Germacrene B	1561	1559	2.7	-	0.7
Azunole	1561	1560	-	-	0.6
(*E*)-Nerolidol	1563	1561	18.4	2.2	0.2
Spathulenol	1578	1577	0.7	0.8	0.1
Globulol	1585	1581	-	-	0.2
Viridiflorol	1593	1591	-	2.2	-
Guaiol	1601	1600	-	0.7	-
α-Cedrol	1601	1601	-	3.6	-
τ-Muurolol	1646	1640	3.4	9.8	6.1
β-Eudesmol	1651	1649	1.3	-	-
α-Cadinol	1654	1652	7.6	24.1	-
α-Bisabolol	1682	1685	1.4	-	2.6
n-Heptadecane	1700	1700	0.7	-	-
Calamenene	1702	1702	0.6	-	-
(*E*,*E*)-Farnesol	1718	1722	1.4	-	-
Benzyl benzoate	1760	1759	0.2	-	-
Benzyl cinnamate	2096	2092	0.4	-	-
Phytol	2125	2 119	0.2	-	-
Hexestrol	2402	2402	-	2.7	-
Total			93.2	97.3	96.7
Monoterpene hydrocarbons			2.2	1.0	15.4
Oxygenated monoterpenes			13.5	2.7	52.0
Sesquiterpene hydrocarbons			41.2	47.5	19.5
Oxygenated sesquiterpenes			34.2	43.4	9.8
Diterpenes			0.2	--	-
Non-terpenes			1.5	2.7	-

[a] Elution order on HP-5MS column; [b] SD Standard deviation, values were insignificant and were omitted from the Table to avoid congestion; RI (Cal.) Retention indices on HP-5MS column; RI (Lit.) Literature retention indices (see Experimental); [c] Pù Mát oil; [d] Tam Đảo oil; - Not identified

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