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Chemical composition of essential oils from the leaves of *Garcinia Gracilis* Pierre (Clusiaceae) and *Canarium parvum* Leenh. (Burseraceae) growing in Vietnam

Nguyen Phuong Hanh, Dinh Thi Thu Thuy, Nguyen Quoc Binh and Isiaka Ajani Ogunwande

Abstract

In continuation of our extensive research on Vietnamese medicinal as they are made available, we report the essential oils from the leaves of *Garcinia Gracilis* Pierre (Clusiaceae) and *Canarium parvum* Leenh. (Burseraceae) of Vietnam which were obtained by hydrodistillation and then analyzed using GC/MS. Thirty-nine compounds were identified in the essential oil of *G. Gracilis*, which was dominated by sesquiterpene hydrocarbon (87.4%). The main sesquiterpene compounds were α -humulene (42.8%), β -caryophyllene (18.4%), α -selinene (8.7%), and β -selinene (7.2%). For *C. Parvum*, 56 compounds were identified, while the major classes of compounds were sesquiterpene hydrocarbons (47.6%) and oxygen-containing sesquiterpenes (33.3%). The major constituents comprised of β -caryophyllene (19.3%), germacrene D (6.6%), caryophyllene oxide (6.0%), α -humulene (5.0%), (*E, E*)- α -farnesene (4.3%) and limonene (4.0%). The essential oil constituents of *G. Gracilis* are being reported for the first time. The compositional pattern of *C. Parvum* showed both qualitative and quantitative variations with a previously analyzed oil sample. The paper discusses further the chemical forms of *Garcinia* and *Canarium* oil samples.

Keywords Medicinal plants, hydrodistillation, essential oil, monoterpenes, sesquiterpenes

1. Introduction

Garcinia is a genus of flowering plants in the family Clusiaceae native to Asia, America, Australia, tropical and southern Africa, and Polynesia. The number of species is disputed; the Kew Gardens recognize up to 400 [1]. *Garcinia Gracilis* Pierre, (Syn. *Garcinia lanceifolia* Roxb.) is a little-known, small, evergreen tree native to tropical Southeast Asia with simple, glossy green leaves and round, red, edible fruit with a tart flavor. The ripe fruits and leaves of *G. Gracilis* are edible [2]. The leaves of this plant have traditionally been used as flavouring materials in foods [2]. The roots are also used as antipyretics folk medicine [3]. Extract from the leaves of *G. Gracilis* displayed superoxide scavenging effects (70.65% inhibition at a concentration of 100 μ g/ml), protection against DNA damage (76.46% at a concentration of 100 μ g/ml), and neuroprotective effects (100% cell viability at a concentration of 100 ng/ml). The extract also exhibited α -glucosidase inhibitory activity (99.49% at a concentration of 2 mg/ml) [4]. A phytochemical screening of the methanolic extract of *G. Gracilis* afforded apigenin-8-C- α -1-rhamnopyranosyl-(1 \rightarrow 2)- β -d-glucopyranoside, 5-hydroxymethyl-2 furaldehyde, and vanillic acid [4]. The first compound showed superoxide anion radical scavenging activity and α -glucosidase inhibitory effects [4]. In Vietnam, 31 *Garcinia* species were found [5]. However, no studies have investigated the chemical constituents and pharmacological activities of essential oils from this plant to date. However, information exists on the essential oil compositions and biological activities of other *Garcinia* plants grown in other parts of the world.

The genus *Canarium* is in the family of Burseraceae in the major group of Angiosperms. *Canarium parvum* Leenh (Syn. *Canarium tonkinense* Guillem.) is a tree or shrub that grows up to 3-8 m tall, and 15-30 cm in diameter. The branchlets are 3-5 mm in diameter while the exstipulate leaves have 2-4 pairs of leaflets [6]. The leaf blades are ovate, elliptic-ovate, or nearly rounded, of dimension 4.5-10 \times 2-5 cm. The male flowers are about 7-10 mm, sparsely pubescent, while the female flowers are about 5.5 mm, nearly glabrescent.

Flowering occurs between November and May, while fruiting takes place from August to November [6]. The native range of this species is China (SE. Yunnan) to Indo-China and Vietnam. The plant is known in Vietnamese as Trám lá nhỏ or Trám chim or bùi. In the only report describing the composition of *C. parvum* essential oil, β -caryophyllene (18.7%), (*E*)- β -ocimene (12.9%), (*Z*)- β -ocimene (11.9%), germacrene D (8.8%) and α -humulene (8.4%) were identified in the leaf while β -caryophyllene (30.4%), α -copaene (20.5%) and (*E*)- β -ocimene (7.7%) were the main compounds found in the stem. However, germacrene D (23.2%), α -amorphene (14.9%), α -copaene (9.8%) and β -elemene (8.6%) were present in the resin [7]. However, while working on a guide to harvest and analyse plants in Đắk Nông Province, Central Highlands, Vietnam, *C. parvum* was collected, which was initially reported to exist only in Ben En National Park, Thanh Hoa Province, Vietnam [7].

In the literature, the chemical compositions and biological activities of essential oils from *Canarium* plants grown in Vietnam and other parts of the world have been described. The leaf of *C. trամdenanum* from Vietnam comprises of β -caryophyllene (16.8%), α -phellandrene (15.9%), γ -elemene (13.1%) and limonene (11.8%), while limonene (25.7%), α -phellandrene (21.7%), α -pinene (12.3%) and β -caryophyllene (10.9%) were present in the stem. However, δ -elemene (14.6%) and bulnesol (16.0%) are the main constituents in the resin [7]. The composition of the essential oil of *C. album* leaves from Vietnam comprised of β -myrcene (23.7%) and β -caryophyllene (15.0%) [8]. Another analyzed sample from Vietnam had a composition pattern made up of β -pinene (33.3%), α -terpinene (19.4%), γ -terpinene (14.1%), and terpinen-4-ol (11.9%) [9]. The predominant compounds of *C. bangalense* were sabinene (15.9%), caryophyllene (17.5%) and *epi*-bicyclosesquiterpene (10.4%) [10].

This paper reports the results of investigation into the chemical constituents of essential oils from the leaves of *G. Gracilis* and *C. parvum* grown in Vietnam are part of the ongoing extensive research aimed at the characterization of the chemical compositions and bioactivities potentials of the plants growing in Vietnam and are poorly described in literature [11-14].

2. Materials and Methods

2.1. Plant Materials: The leaves of *G. Gracilis* and *C. parvum* were collected in April 2019 from Đắk Nông Province, Central Highlands, Vietnam. The amounts of leaf sample collected were about 1860 g for *G. Gracilis* and about 1630 g for *C. parvum*. Voucher specimen NPH1.2019 and NPH 2019, respectively, were deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

2.2. Extraction of essential oils from *G. Gracilis* and *C. Parvum*: For the extraction of the essential oils, 1860 g of the leaves of *G. Gracilis* and 1630 g of *C. parvum* were used. Hydrodistillation was carried out in a 5-L glass Clevenger-type apparatus for 4 h. After distillation, the essential oils were collected, dried over anhydrous sodium sulfate, and kept in dark glass bottles at 4 °C before analysis. The oil yields were calculated on a dried weight basis. The collected oil sample was light-yellow colored, with pungent and slightly bitter taste.

2.3. Gas chromatography/mass spectroscopy (GC/MS) analysis: Gas chromatography (GC) analysis was performed on an Agilent Technologies HP7890A GC equipped with a

HP5-MS column (60 m \times 0.25 mm, film thickness 0.25 μ m, Agilent Technologies). The analytical conditions include helium as a carrier gas at a flow rate of 1 mL/min; the injector and detector temperatures set at 250 and 280 °C, respectively; while the column temperature was programmed from 60 °C to 240 °C at 4 °C/min. Essential oil samples were injected by splitting. The split ratio was 100: 1. The volume injected was 1 μ L of diluted essential oils in Dimethylsulfoxide as described previously [11-14].

Gas chromatography/mass spectroscopy (GC/MS) analysis: The instrument used for the GC/MS analysis consists of a HP7890A GC fitted with mass spectrum detector (MSD, Agilent Technologies HP5975C) and equipped with a HP5-MS column (60 m \times 0.25 mm, film thickness 0.25 μ m). The GC conditions were the reported as above. The mass spectra in electron mode were generated at 70 eV, acquisitions scan mass range of 45-450 amu under full scan and emission current of 40 mA, at a sampling rate of 1.0 scan/s as described previously [11-14].

A homologous n-alkane series was used as the standard to calculate the retention indices (RI) of each component. Relative amounts of individual components were calculated based on the GC peak areas (MSD response). MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook [15] was used to match mass spectra and retention indices.

2.4. Chemical compositions of the essential oils of *G.*

***Gracilis*:** The yield of the essential oil of *G. Gracilis* was 0.09% calculated on a dried weight basis. The main class of compounds identified in *G. Gracilis* essential oil are the sesquiterpene hydrocarbons (87.4%). Other classes of compounds in the essential oil including oxygenated monoterpenes (0.4%), oxygenated sesquiterpenes (6.5%), diterpenes (1.3%), di-nor-sesquiterpenoid (0.2%) and non-terpenes (0.5%) were present in much lower amount (Table 1). Monoterpene hydrocarbon compounds were not identified in the leaf essential oil of *G. Gracilis*. The main compounds of the essential oils were α -humulene (42.8%), β -caryophyllene (18.4%), α -selinene (8.7%) and germacrene D (7.2%). All other compounds namely α -copaene (2.3%), β -eudesmol (2.2%), δ -cadinene (1.2%), β -chamigrene (1.6%), 9-*epi*-*E*-caryophyllene (1.1%), and phytol (1.1%) were identified in amount \geq 1%. This is the first report on the chemical constituents of essential oils from *G. Gracilis*. The major chemical compounds of essential oils from some other *Garcinia* plants reported so far are shown in Table 2.

A comparative analysis of the present data with the main constituents of essential oils from some other *Gracilis* plants [16-28] indicated that monoterpene compounds were rarely described in the essential oils. The major compounds identified in these essential oils were mainly sesquiterpene compounds (Table 2). However, non-terpene compounds such as palmitoleic acid and palmitic in the stem bark essential oils of *G. Celebica* from Malaysia [18], *n*-undecane from the leaves of *G. Travanconica* grown in India [22] and citronellic acid from *G. kola* analysed from Nigeria [28] were also reported. Due to the high contents of α -humulene and β -caryophyllene, it can be postulated that the chemical composition of essential oil of *G. Gracilis* from Vietnam belongs to the chemical group similar to *G. atroviridis* from India [20], *G. huillensis* from Zimbabwe [21], as well as *G. Indica*, *G. Imbertii*, *G. Talbotii*, *G. Pushpangadaniana*, and *G. Rubro-echinata* from India [22].

Table 1: Chemical compositions of the leaf essential oils of *C. parvum* and *G. Gracilis* collected in Vietnam

Sr. No.	RT (min)	Compounds ^a	RI ^b	RI ^c	<i>G. Gracilis</i>	<i>C. parvum</i>
1	10.13	α -Pinene	939	932	-	0.6
2	11.74	Myrcene	992	990	-	0.2
3	12.73	α -Terpinene	1022	1014	-	0.2
4	13.01	o-Cymene	1030	1020	-	0.7
5	13.15	Limonene	1034	1028	-	4.0
6	14.15	γ -Terpinene	1063	1056	-	0.3
7	15.51	Linalool	1103	1100	0.1	-
8	15.60	n-Nonanal	1106	1106	0.3	0.3
9	16.10	p-Ethylanisol	1120	1121	0.2	-
10	17.60	(2E)-Nonen-1-al	1162	1164	-	0.2
11	18.46	Terpinen-4-ol	1187	1187	-	1.2
12	18.90	α -Terpineol	1200	1200	0.3	0.4
13	19.07	Methyl salicylate	1204	1206	-	0.2
14	24.35	α -Cubebene	1360	1363	-	0.3
15	25.31	α -Copaene	1389	1387	2.3	2.5
16	25.74	β -Cubebene	1402	1400	-	0.5
17	25.78	trans- β -Elemene	1403	1401	-	0.7
18	26.46	α -Gurjunene	1425	1422	-	0.2
19	26.47	cis- α -Bergamotene	1426	1425	0.2	-
20	26.87	β -Caryophyllene	1437	1437	18.4	19.3
21	27.09	β -Copaene	1445	1447	0.1	0.3
22	27.11	trans- α -Bergamotene	1446	1446	0.1	-
23	27.29	α -Guaiene	1451	1453	0.2	-
24	27.46	Guaiene-6,9-diene	1457	1457	0.3	-
25	27.70	allo-Aromadendrene	1464	1464	0.9	-
26	27.93	α -Humulene	1472	1472	42.8	5.0
27	28.15	9- <i>epi</i> -E-Caryophyllene	1479	1480	1.1	0.5
28	28.39	4,5-di- <i>epi</i> -Aristolochene	1486	1486	0.3	-
29	28.44	trans-Cadina-1(6),4-diene	1488	1486	-	0.3
30	28.50	β -Chamigrene	1490	1489	1.6	-
31	28.53	γ -Muurolole	1491	1489	-	0.9
32	28.71	(E)- β -Ionone	1496	1496	0.2	-
33	28.77	Germacrene D	1498	1498	-	6.6
34	28.96	β -Selinene	1505	1503	7.2	1.0
35	29.19	(E,E)- α -Farnesene	1512	1513	-	4.3
36	29.21	α -Selinene	1513	1515	8.7	-
37	29.34	β -Bisabolene	1518	1518	0.3	-
38	29.44	α -Bulnesene	1521	1521	0.3	-
39	29.72	γ -Cadinene	1530	1528	0.3	0.6
40	29.92	δ -Cadinene	1537	1537	1.2	2.9
41	29.97	cis-Calamenene	1539	1541	0.3	0.2
42	30.09	(E)- α -Bisabolene	1542	1543	0.2	-
43	30.42	α -Cadinene	1553	1555	0.2	0.3
44	30.62	α -Calacorene	1560	1561	0.4	0.8
45	30.76	Occidentalol	1565	1568	-	0.3
46	30.93	(E)-Nerolidol	1571	1573	0.2	0.8
47	31.31	Dendrolasin	1583	1585	-	0.2
48	31.42	Mintoxide	1587	1589	-	0.5
49	31.58	Caryophyllene alcohol	1592	1590	0.8	0.6
50	31.77	Spathulenol	1599	1600	-	2.5
51	31.96	Caryophyllene oxide	1605	1608	0.3	6.0
52	32.07	Clovenol	1609	1612	-	0.8
53	32.22	Viridiflorol	1614	1616	-	2.1
54	32.38	Humulene epoxide I	1620	1622	0.5	0.4
55	32.59	Cedrol	1627	1630	0.3	1.1
56	32.71	Humulene epoxide II	1630	1634	0.8	2.2
57	33.15	1- <i>epi</i> -Cubenol	1647	1647	-	1.0
58	33.27	γ -Eudesmol	1652	1650	0.8	0.2
59	33.46	Caryophylla-3(15),7(14)-dien-6-ol	1658	1660	-	1.0
60	33.50	<i>epi</i> - α -Cadinol	1660	1662	-	1.6
61	33.54	<i>epi</i> - α -Muurolol	1661	1663	-	2.5
62	33.63	α -Muurolol	1664	1666	-	1.0
63	33.89	β -Eudesmol	1673	1672	2.2	-
64	33.92	α -Cadinol	1674	1674	-	3.5

65	33.95	α -Eudesmol	1675	1676	0.1	-
66	34.02	neo-Intermedeol	1678	1678	-	3.0
67	34.27	(E)-Asarone	1687	1688	-	0.5
68	34.39	9- <i>epi</i> -E-14-Hydroxycaryophyllene	1691	1692	-	1.2
69	38.49	Hexahydrofarnesylacetate	1845	1856	0.5	-
70	34.50	Cadalene	1695	1700	-	0.4
71	35.11	Pentadecanal	1717	1717	-	0.8
72	37.84	14-Hydroxy- δ -Cadinene	1820	1824	-	0.3
73	41.16	Isophytol	1951	1962	0.2	-
74	45.09	Phytol	2117	2119	1.1	0.3
Total					95.95	90.3
Monoterpene hydrocarbons (Sr. No: 1-6)					-	6.0
Oxygenated monoterpenes (Sr. No: 7, 11-12)					0.4	1.6
Sesquiterpene hydrocarbons (Sr. No: 14-31, 33-44, 70)					87.4	47.6
Oxygenated sesquiterpenes (Sr. No: 45-66, 69, 72)					6.5	32.8
Diterpenes (Sr. No: 73, 74)					1.3	0.3
Non-terpenes (Sr. No: 8, 9, 10, 71)					0.5	1.3
Benzoate ester (Sr. No: 13)					-	0.2
Di-nor-sesquiterpenoid (Sr. No: 32)					0.2	-
Phenylpropanoid (Sr. No: 67)					-	0.5

^a Elution order on HP-5MS column; ^b Experimental retention indices; ^c Literature retention indices on HP-5MS column as seen in NIST¹⁵; ^d means of three replicate values, SD (\pm) omitted to avoid congestion; Sr. No, serial number; - not identified.^a

A comparative analysis of the present data with the main constituents of essential oils from some other *Gracilis* plants [16-28] indicated that monoterpene compounds were rarely described in the essential oils. The major compounds identified in these essential oils were mainly sesquiterpene compounds (Table 2). However, non-terpene compounds such as palmitoleic acid and palmitic in the stem bark essential oils of *G. Celebica* from Malaysia [18], *n*-undecane from the leaves

of *G. Travancorica* grown in India [22] and citronellic acid from *G. kola* analysed from Nigeria [28] were also reported. Due to the high contents of α -humulene and β -caryophyllene, it can be postulated that the chemical composition of essential oil of *G. Gracilis* from Vietnam belongs to the chemical group similar to *G. huillensis* from Zimbabwe [21], *G. nigrolineata* from Malaysia [25].

Table 2: Chemical forms of the leaf essential oils of some *Garcinia* plants reported in literature

Chemical forms	Species	Origin	References
α -copaene	<i>G. Celebica</i>	Malaysia	16
	<i>G. Pedunculata</i>	India	17
(E)- β -farnesene / β -caryophyllene	<i>G. Atroviridis</i>	Malaysia	18
	<i>G. Gummi-Gutta</i>	"	19
	<i>G. Huillensis</i>	Zimbabwe	21
	<i>G. Indica</i>	India	22
	<i>G. Imbertii</i>	"	22
	<i>G. Talbotii</i>	"	22
	<i>G. Rubro-Echinata</i>	"	22
β -caryophyllene	<i>G. Mangostana</i>	Nigeria	23
	<i>G. Gardneriana</i>	Brazil	26
	<i>G. Morella</i> Var. <i>Mucrona</i>	India	17
	<i>G. Assamica</i>	"	17
β -caryophyllene/ α -humulene/valencene	<i>G. Xanthochymus</i>	"	17
	<i>G. Huillensis</i>	Zimbabwe	21
<i>trans</i> - α -bergamotene/ β -curcumene	<i>G. Nigrolineata</i>	Malaysia	25
<i>trans</i> - α -bergamotene/ β -curcumene	<i>G. Gardneriana</i>	Brazil	24
β -caryophyllene/ α -selinene/ β -selinene	<i>G. Indica</i>	India	22
β -selinene/ β -caryophyllene	<i>G. Lanceifolia</i>	India	17
α -cedrene/chamigrene	<i>G. Gardneriana</i>	Brazil	26
germacrene D/ β -caryophyllene	<i>G. Dulcis</i>	India	17
δ -cadinene/ α -copaene	<i>G. Gummi-Gutta</i>	India	22
β -copaene/ α -humulene	<i>G. Morella</i>	"	22
bicyclogermacrene/ β -caryophyllene	<i>G. Wightii</i>	"	22
hexahydrofarnesylacetate	<i>G. Imbertii</i>	"	22
	<i>G. Travancorica</i>	"	22
(-)- β -caryophyllene/ β - caryophyllene alcohol/ α -humulene	<i>G. Atroviridis</i>	Malaysia	20
(-)- β -caryophyllene/ β - caryophyllene alcohol/ α -humulene	"	;;	20
palmitoleic acid/palmitic acid	"	"	18
δ -cadinene/ γ -cadinene/ γ -muurolene/ β -caryophyllene	<i>G. Pushpangadaniana</i>	India	22
<i>n</i> -undecane	<i>G. Travancorica</i>	India	22

2.5. Chemical compositions of the essential oils of *C. parvum*:

A look at Table 1 showed that 56 compounds were identified in the present essential oil of *C. parvum* accounting for 90.3% of the total oil contents. The yield of the essential oil was 0.04%. The compositional pattern comprised of monoterpene hydrocarbons (6.0%), oxygenated monoterpenes (1.6%), sesquiterpene hydrocarbons (47.6%), oxygenated sesquiterpenes (32.8%), benzoate ester (0.2%), phenylpropanoids (0.5%), diterpene (0.3%) and aliphatic aldehydes (1.3%). The main compounds of the essential oil were β -caryophyllene (19.3%), germacrene D (6.6%), caryophyllene oxide (6.0%), α -humulene (5.0%), (*E*, *E*)- α -farnesene (4.3%) and limonene (4.0%). Other significant constituents of the essential oil include α -cadinol (3.5%), *neo*-intermedeol (3.0%), δ -cadinene (2.9%), α -copaene (2.5%), spathulenol (2.5%), *epi*- α -muurolol (2.5%), and viridiflorol (2.1%). A comparative analysis of the present study with a previous report on the compositional pattern of essential oil of

C. parvum showed some interesting observations. Firstly, β -caryophyllene and germacrene D, the main compounds of the previously reported constituents of *C. parvum* essential oil [7], were also identified likewise in the present investigated oil sample. However, the content of caryophyllene oxide in the present sample is higher than reported previously. In addition, (*E*)- β -ocimene and α -amorphene reported previously as part of the constituents of *C. parvum* essential oil [7], were not identified in the present study. In addition, several other compounds such as (*Z*)- β -ocimene, methyl salicylate, p-cymen-7-ol, germacrene B, *endo*-bourbonalol, vulgareol B, and acorenone B, among others that were present in the previously analyzed samples were not detected in this study. Conversely, some compounds such as mintoxide, dendrolasin, *neo*-intermedeol, (*E*)-asarone, 9-*epi*-*E*-14-hydroxycaryophyllene, among others present in the present investigated oil sample of *C. parvum*, were not reported previously among the constituents of the essential oil.

Table 3: Chemical forms of essential oils of some *Canarium* plants reported in literature

Chemical forms	Species	Origin	References
β -caryophyllene / (<i>E</i>)- β -ocimene/ (<i>Z</i>)- β -ocimene	<i>C. Parvum</i>	Vietnam	7
β -caryophyllene/ α -copaene	"	"	7
germacrene D/ α -amorphene	"	"	7
β -caryophyllene/ α -phellandrene/ γ -elemene/ limonene	<i>C. Tramdenanum</i>	"	"
limonene/phellandrene/ α -pinene/ β -caryophyllene	"	"	"
δ -elemene/bulnesol	"	"	"
β -myrcene/ β -caryophyllene	<i>C. Album</i>	"	8
β -pinene/ α -terpinene	"	"	9
γ -terpinene/terpinen-4-ol	"	"	9
sabinene/caryophyllene/ <i>epi</i> -bicyclosesquiterpene	<i>C. Bangalense</i>	"	10
Limonene	<i>C. Schweinfurthii</i>	Cameroon	29
	"	Gabon	30
	"	Nigeria	31
	"	Cote D'ivoire	32
	<i>C. Luzonicum</i>	Turkey	33
	"	Slovakia	34
	<i>C. Strictum</i>	Pakistan	35
	<i>C. Album</i>	Japan	36
	<i>C. Pimela</i>	China	37
	(<i>E</i>)-caryophyllene/germacrene B	<i>C. Schweinfurthii</i>	Nigeria
γ -terpinene/ α -phellandrene	"	Uganda	38
α -thujene / β -phellandrene	"	"	38
α -terpineol	"	Nigeria	31

Conclusions

This study showed that the composition of hydrodistilled essential oil of *G. Gracilis* are being reported for the first time, containing α -humulene (42.8%), β -caryophyllene (18.4%), while *C. parvum* of Highlands, Dāk Nong Province, Vietnam, composed mainly of β -caryophyllene (19.3%), germacrene D (6.6%) and caryophyllene oxide (6.0%). The compositional pattern of *C. parvum* showed both qualitative and quantitative variations with a previously analyzed oil sample.

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