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Volatile constituents of *Atalantia roxburghiana* Hook. f., *Tetradium trichotomum* Lour. and *Macclurodendron oligophlebia* (Merr.) Hartl. (Rutaceae) from Vietnam

[Compuestos volátiles de *Atalantia roxburghiana* Hook. f., *Tetradium trichotomum* Lour. y *Macclurodendron oligophlebia* (Merr.) Hartl. (Rutaceae) provenientes de Vietnam]

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Abstract: The chemical constituents of essential oils obtained by hydrodistillation of the leaves of *Atalantia roxburghiana* Hook. f. and *Tetradium trichotomum* Lour., as well as the leaves and fruits of *Macclurodendron oligophlebia* (Merr.) Hartl. (Rutaceae) are being reported. The essential oils were analysed by using gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). Sabinene (36.9%) was the most singly abundant compound in the leaf of *A. roxburghiana*. The major constituents present in the leaf oil of *T. trichotomum* were (E)- β -ocimene (24.8%), α -pinene (10.4%), (Z)- β -ocimene (9.4%) and β -caryophyllene (8.0%). On the other hand, while α -pinene (17.5%), β -caryophyllene (15.5%) and caryophyllene oxide (10.6%) occurred in higher proportion in the leaf of *M. oligophlebia*, the fruit oil was dominated by benzyl benzoate (16.8%), (E, E)-farnesol (8.3%) and β -caryophyllene (6.0%).

Keywords: *Atalantia roxburghiana*, *Macclurodendron oligophlebia*, *Tetradium trichotomum*, hydrodistillation, sabinene, (E)- β -ocimene, benzyl benzoate

Resumen: Se muestran los constituyentes químicos de los aceites esenciales obtenidos, por hidrodestilación, de las hojas de *Atalantia roxburghiana* Hook. f. y de *Tetradium trichotomum* Lour., así como de las hojas y frutos de *Macclurodendron oligophlebia* (Merr.) Hartl. (Rutaceae). Los aceites esenciales fueron analizados por Cromatografía de Gases (CG) y por Cromatografía de Gases acoplada a Espectrometría de Masas (CG-EM). El compuesto más abundante en las hojas de *A. roxburghiana* es el sabineno (36.9%); mientras que los mayoritarios en el aceite de las hojas de *T. trichotomum* fueron (E)- β -ocimeno (24.8%), α -pineno (10.4%), (Z)- β -ocimeno (9.4%) y β -cariofileno (8.0%). En las hojas de *M. oligophlebia* los compuestos más abundantes fueron α -pineno (17.5%), β -cariofileno (15.5%) y óxido de cariofileno (10.6%); sin embargo, en el aceite obtenido del fruto fueron benzoato de bencilo (16.8%), (E, E)-farnesol (8.3%) y β -cariofileno (6.0%).

Palabras clave: *Atalantia roxburghiana*, *Macclurodendron oligophlebia*, *Tetradium trichotomum*, hidrodestilación, sabineno, (E)- β -ocimeno, benzoato de bencilo

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INTRODUCCIÓN

In continuation of an extensive research on the analysis of the chemical constituents of relatively poor studied species of Vietnamese flora (Chau *et al.*, 2015; Huong *et al.*, 2017), we report herein the chemical compounds identified in the hydrodistilled essential oils of *Atalantia roxburghiana* Hook.f., *Macclurodendron oligophlebia* (Merr.) Hartl. and *Tetradium trichotomum* Lour. collected from B n En National Park, Thanh H a Province, Vietnam.

Atalantia roxburghiana is a small tree which grows up to 10 m high and with little or no spines. The leaves are oval shape up to long 9 - 15 cm long with bulging veins and tendons on both sides. The flowers are in clusters, 4cm long while the fruit is spherical like tiny oranges with diameter of 1- 2.5 cm. Flowering occur in April while the edible fruits are produced from June to August. The leaves used in medicine to treat respiratory diseases. Decoctions of leaves are used to lung disorders (Wiert, 2006). The chloroform extract of *A. roxburghiana* was reported to exert muscle relaxant through inhibition of calcium influx through the calcium channel of the cell membrane (Rashid *et al.*, 1995). Extracts of the plant has been used as antidote for snake venoms. (Suthari & Raju, 2016). The plant contains an alkaloid N-methylflindersine (Baxter *et al.*, 1998). Only one report could be found in literature in which the main constituents of the essential oil obtained from the branches and leaves of *A. roxburghiana* (Minh *et al.*, 2010) were identified as γ -terpinene (38.3%) and p-cymene (15.7%)

Tetradium trichotomum is a shrub or tree that grows up to 8 m tall. The foliage leaves are about 12-37 cm in diameter. It has about 4 or 5 flowers with green petals which turn brownish at maturity. The globose seed are edible. Flowering occurs between April and August while fruiting takes place from September to November. A phytochemical examination of the stem and root bark of *T. trichotomum* yielded the alkaloids α -allocryptopine and dictamnine and the limonoids limonin, limonexic acid and calodendrolide (Quader *et al.*, 1990). *Tetradium trichotomum* is regarded as a synonym of *Euodia trichotoma* Lour (But *et al.*, 2009). Previously, *cis*- β -ocimene (18.7%) and *trans*- β -ocimene (48.1%) were the major constituents identified in the essential oil of the leaf of *E. trichotomum* (Thang *et al.* 2006).

Macclurodendron oligophlebia is an

evergreen tree about 16 m high and 30-40 cm in diameter of. The veined bark is pale gray while the monad leaves grow as ovate rounded rectangular form, about 7-18 cm long. The white blue fragrant flowers are bisexual in width from 3.5 to 7 cm. Flowering starts in June (H ng *et al.*, 2015). There is no information on the chemical constituents of the volatile and non-volatile extracts as well as biological activity of the *M. oligophlebia*.

This paper therefore report the chemical compounds identified in the essential oils of *A. roxburghiana*, *M. oligophlebia* and *T. trichotomum* and provide further information on the diversity of the volatile components of Vietnamese flora.

MATERIALS AND METHOD

Plants collection

Samples of *A. roxburghiana*, *M. oligophlebia* and *T. trichotomum* were collected from B n En National park, Thanh H a Province, Vietnam, in August 2013. Botanical identifications were carried out by Curators at Herbarium of Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Vietnam. Voucher specimens HVC 375, HVC 432 and HVC 433 respectively were deposited at the Herbarium Vietnam. Plant samples were air-dried prior to extraction.

Distillation of the essential oils

Briefly, 500 g each of the air-dried pulverized samples were carefully introduced into a 5 L flask and distilled water (5 L) was added until it covers the sample completely. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification (Vietnamese Pharmacopoeia, 1997). The oils were kept under refrigeration (4  C) until the moment of analyses.

Analysis of the essential oils

GC analysis of the oils were done with Agilent HP 6890 Plus instrument equipped with HP-5MS column (30 m x 0.25 mm; 0.25 μ m film thickness) and N₂ as the carrier gas with a flow rate of 1 mL/min. The sample injection (1 μ L) was done with 10:1 split ratio. The injection port temperature was 250  C. The column oven temperature was initially set to 40  C and held constant for 2 min, then raised to 220  C at rate of 4  C/min. The temperature was kept constant at 220  C for 10 min. 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. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

GC/MS analysis of the oils were performed with Agilent Technologies HP 6890N Plus Chromatograph fitted with HP-5MS column (30 m x 0.25 mm; 0.25 μ m film thickness) and interfaced with a mass spectrometer HP 5973 MSD. The conditions were the same as described above for GC. The MS was operated with ionization voltage 7 of 0

eV while the emission current was set at 40 mA. The acquisitions scan mass range of 35-350 amu was used at a sampling rate of 1.0 scan/s.

Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST (NIST, 2011).

Table 1
Chemical constituents of essential oils of *A. roxburghiana*, *M. oligophlebia* and *T. trichotomum*

Compound ^b	RI (Calculate)	RI (Literature)	Percentage composition ^a			
			<i>A. rl</i>	<i>M. ol</i>	<i>M. of</i>	<i>T. tl</i>
α -Thujene	930	921	1.2	-	-	-
α -Pinene	939	932	2.1	17.5	3.6	10.4
Camphene	953	946	0.1	0.2	-	0.2
Sabinene	976	967	36.9	0.8	0.8	-
β -Pinene	980	978	-	-	1.1	0.7
β -Myrcene	990	988	2.4	0.6	0.8	1.9
n-Nonane	1000		-	-	-	1.1
α -Phellandrene	1006	1004	0.3	0.1	1.3	-
δ -3-Carene	1011	1007	0.7	-	-	-
α -Terpinene	1017	1014	2.2	-	0.5	-
<i>o</i> -Cymene	1024	1021	0.5	-	-	-
Limonene	1032	1030	-	1.4	4.7	0.9
(<i>Z</i>)- β -Ocimene	1043	1034	0.1	3.0	1.0	9.4
(<i>E</i>)- β -Ocimene	1052	1044	0.5	4.9	0.8	24.8
γ -Terpinene	1061	1056	3.7	0.2	0.9	-
<i>cis</i> -Sabinene hydrate	1070	1071	0.3	-	-	-
α -Terpinolene	1090	1089	0.9	0.3	-	-
Linalool	1100	1095	-	0.2	1.2	0.7
<i>allo</i> -Ocimene	1128	1128	0.1	1.4	-	5.0
Terpinene-1-ol	1139	1134	0.1	-	-	-
Camphor	1145	1141	-	-	0.7	-
<i>allo</i> -neo-Ocimene	1147	1143	-	1.0	2.4	0.3
Borneol	1167	1167	-	-	1.9	-
Terpinen-4-ol	1177	1177	0.7	-	0.9	-
Decanal	1200	1200	-	0.1	-	-

(<i>E, E</i>)-2,6-Dimethyl-3,5,7-octatriene-2-ol ^d	1209	1208	-	-	-	0.7
Geraniol	1253	1254	0.1	-	-	-
Bornyl acetate	1289	1287	0.1	-	-	-
2-Undecanone	1291	1294	-	-	-	3.9
Bicycloelemene	1327	1337	3.7	3.0	-	2.4
α -Cubebene	1351	1345	0.1	0.4	-	0.2
α -Ylangene	1375	1372	0.1	-	-	-
α -Copaene	1377	1374	0.2	3.1	-	0.2
β -Cubebene	1388	1387	-	-	-	0.2
β -Elemene	1391	1389	0.6	0.9	1.1	5.7
α -Gurjunene	1412	1409	2.7	0.3	-	-
β -Caryophyllene	1419	1417	6.1	15.5	6.0	8.0
β -Gurjunene	1434	1431	-	-	0.9	-
<i>trans</i> - α -Bergamotene	1435	1435	-	1.5	-	-
γ -Elemene	1437	1437	1.0	-	-	-
Aromadendrene	1441	1439	1.3	1.0	-	0.1
α -Humulene	1454	1452	1.3	2.4	2.1	1.3
Ishwarane ^d	1467	149	0.6	-	-	-
γ -Gurjunene	1477	1477	-	0.8	-	-
γ -Muurolene	1480	1484	0.5	-	-	-
<i>ar</i> -Curcumene	1481	1483	1.7	-	-	-
Germacrene D	1485	1485	-	0.7	1.1	1.1
α -Amorphene	1485	1485	-	-	2.4	0.3
β -Selinene	1486	1486	-	0.2	4.4	-
<i>epi</i> -Bicyclosesquiphellandrene	1489	1488	1.7	-	-	-
Zingiberene	1494	1493	1.5	-	-	-
Cadina-1,4-diene	1496	1496	-	1.7	1.2	-
Valencene	1496	1498	-	-	-	0.3
Bicyclogermacrene	1500	1500	2.7	3.6	-	3.3
α -Muurolene	1500	1500	-	-	1.4	-
(<i>E, E</i>)- α -Farnesene	1508	1505	-	0.8	-	1.3
γ -Cadinene	1514	1514	-	0.5	-	0.2
Calamenene	1521	1521	-	-	1.5	-
δ -Cadinene	1525	1522	0.5	1.9	1.5	1.0
Selina-4(15), 7(11)-diene	1534	1532	0.2	-	-	-
Cadina-1(2),4-diene ^d	1539	1532	-	3.8	-	-
α -Agarofuran	1541	1540	-	0.2	-	-
Calacorene	1546	1541	-	-	0.9	-
Selina-3, 7(11)-diene	1547	1545	-	1.0	-	-
Elemol	1550	1548	-	-	-	0.2

Ledol	1561	1560	-	-	-	0.4
Germacrene B	1561	1562	0.5	-	-	-
(<i>E</i>)-Nerolidol	1563	1561	0.6	-	-	0.6
Spathulenol	1578	1577	0.6	-	1.1	2.3
Caryophyllene oxide	1583	1581	0.4	10.6	1.1	1.5
Viridiflorol	1593	1591	-	0.5	-	-
α -Guaiol	1600	1600	-	1.8	1.5	-
β -Oplophenone	1608	1608	-	-	1.5	-
Caryophyllenol	1611	1606	-	2.8	-	-
3,6-Dimethylpiperazine-2,5-dione ^d	1612		7.6	-	-	-
β -Eudesmol	1651	1651	0.8	-	2.7	-
α -Cadinol	1654	1652	-	-	4.7	2.3
Bulnesol	1672	1672	-	-	2.7	-
α -Bisabolol	1685	1685	-	0.6	-	-
(<i>E, E</i>)-Farnesol	1718	1722	-	0.5	8.3	1.4
Benzyl benzoate	1760	1759	-	-	16.8	4.4
Farnesyl acetate ^c	1846	1842	-	-	3.8	-
Phytol	2125	2119	-	0.3	-	-
Total			90.0	92.1	92.2	98.7
Monoterpene hydrocarbons			51.7	31.4	17.9	53.6
Oxygenated monoterpenes			1.3	0.2	4.7	0.7
Sesquiterpene hydrocarbons			23.3	43.3	24.4	25.6
Oxygenated sesquiterpenes			2.4	16.8	45.2	13.1
Diterpenes			-	0.3	-	-
Non-terpenes			11.7	0.1	-	5.7

^aStandard Deviation (SD \pm) were insignificant and excluded from the Table to avoid congestion

^bElution order on HP-5MS column

^cCorrect isomer not identified

RI (Cal.) Retention indices on HP-5MS column

RI (Lit.) Literature retention indices

- Not identified

^dMode of identification, retention indices, mass spectrum and co-injection

A. rl, *A. roxburghiana* (leaf); M. ol, *M. oligophlebia* (leaf)

M. of, *M. oligophlebia* (fruit); T. tl, *T. trichotomum* (leaf)

RESULTS AND DISCUSSION

The yield of essential oils were 0.35% (v/w, *A. roxburghiana*), 0.43% and 0.71% (v/w, *M. oligophlebia* leaf and fruit respectively) and 1.22% (v/w, *T. trichotomum*), calculated on a dry weight basis. Oil samples were light yellow in colouration. Table 1 indicated the percentages and identities of compounds present in the oils. From Table 1, monoterpene hydrocarbons (51.7%) and sesquiterpe-

ne hydrocarbons (23.4%) were the main classes of compounds present in the leaf oil of *A. roxburghiana*. The oxygenated terpenoids are present in lower quantity. Sabinene (36.9%) was the most singly abundant compound in the leaf of *A. roxburghiana* while 3,6-dimethylpiperazine-2,5-dione (7.6%) and β -caryophyllene (6.1%) were present in significant amount. The main constituents previously identified in the oils of *A. roxburghiana* (Minh et al., 2010)

were γ -terpinene (38.3%) and p-cymene (15.7%). These compounds were either identified in lower quantity (γ -terpinene) or absent (p-cymene) in the present oil sample. Moreover, sabinene was not a significant compound of the previous analysis.

However, sabinene has been described as a quantitatively significant compound of essential oils of *Atalantia* species such as *A. monophylla* (Sathya et al., 2002; Das & Swamy, 2013; Thirugnanasampandan et al., 2015; Nattudurai et al., 2016) and *A. guillauminii* (Hùng et al., 2016) and therefore, may be of chemotaxonomic importance.

It could be seen that monoterpene hydrocarbons (53.6%), sesquiterpene hydrocarbons (25.6%) and oxygenated sesquiterpenes (13.1%) represent the abundant class of compounds in *T. trichotomum*. The major constituents present in the leaf oil of *T. trichotomum* were (*E*)- β -ocimene (24.8%), α -pinene (10.4%), (*Z*)- β -ocimene (9.4%) and β -caryophyllene (8.0%). Although, *cis*- β -ocimene and *trans*- β -ocimene were described previously from the essential oil of a synonym of the plant (*E. trichotomum*), these compounds were also present in significant amount in *T. trichotomum* oil sample. However, some other compounds such as α -pinene and β -caryophyllene that were present in the investigated oil were not identified in the previous sample. The essential oil from the fruits of another species *T. glabrifolium* (Liu et al., 2015) was found to contain 2-tridecanone (43.38%), 2-undecanone (24.09%) and D-limonene (13.01%). The contents of D-limonene and 2-undecanone in the present oil were insignificant while 2-tridecanone was not identified. This may be attributed to the differences in the nature of the plant and the plant's part being analysed.

The leaf oil of *M. oligophlebia* comprised mainly of monoterpene hydrocarbons (31.4%), sesquiterpene hydrocarbons (43.3%) and oxygenated sesquiterpenes (16.8%). α -Pinene (17.5%), β -caryophyllene (15.5%) and caryophyllene oxide (10.6%) occurred in higher proportion in the leaf of *M. oligophlebia*. On the other hand monoterpene hydrocarbons (17.9%), sesquiterpene hydrocarbons (24.4%) and oxygenated sesquiterpenes (45.2%) were the main classes of compounds identified in the fruit oil. The quantitatively significant constituents of the oil were benzyl benzoate (16.8%), (*E, E*)-farnesol (8.3%) and β -caryophyllene (6.0%). The authors are not aware of any previous study on the essential oil of this species or any other species in the genus. Therefore, the present result may represent the first of

its kind aimed at the characterization of the volatile constituents of *M. oligophlebia*.

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