© 2015
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 14 (1): 60 - 66
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Composition of essential oils from *Euodia lepta* (Spreng.) Merr and *Euodia calophylla* Guill., grown in Vietnam

[Composición de los aceites esenciales de *Euodia lepta* (Spreng.) Merr y *Euodia calophylla* Guill., crecidas en Vietnam]

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Abstract: The chemical compositions of essential oils obtained by hydrodistillation of the leaves, stems and flowers of *Euodia lepta* and *Euodia callophylla* grown in Vietnam and then analysed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS) were being reported. The main compounds of the leaves oil of *E. lepta* were (E)-β-ocimene (24.4%), α-pinene (9.8%), (Z)-β-ocimene (6.3%) and δ-cadinene (5.2%), while the stems oil comprised of spathulenol (26.0%), (E)-β-ocimene (9.9%) and (Z)-9-octadecenamide (7.7%). However, cis-carane (19.2%), α-cadinol (10.8%), α-pinene (10.5%) and (E)-β-ocimene (9.0%) were present in the flowers oil of *E. lepta*. On the other hand, α-pinene (8.3%), trans-α-bergamotene (7.5%), (E)-β-ocimene (7.0%) and (E)-nerolidol (6.6%) were the major constituents of the leaves oil of *E. calophylla*. The quantitatively significant compounds of the stems oil were (E,E)-α-farnesene (11.9%), α-terpinolene (11.3%) and α-pinene (8.2%), while α-pinene (21.6%), limonene (19.0%) and sabinene (15.5%) were obtained from the flowers oil.

Keywords: *Euodia lepta*, *Euodia callophylla*, essential oil, monoterpenes, sesquiterpenes

Resumen: La composición químicas de los aceites esenciales obtenidos por hidrodestilación de las hojas, tallos y flores de *Euodia lepta* y *Euodia callophylla* cultivadas en Vietnam, fueron analizados por cromatografía de gases-detector de ionización de llama (GC-FID) y la cromatografía de gases/espectrometría de masas (GC-MS). Los principales compuestos del aceite de hojas de *E. lepta* fueron (E) -β-ocimeno (24,4%), α-pineno (9,8%), (Z)-β- ocimeno (6,3%) y δ-cadineno (5.2%), mientras que los tallos de aceite estaban compuestos de spatulenol (26,0%), (E) -β-ocimeno (9,9%) y (Z) -9- octadecenamida (7,7%). Sin embargo, cis-carano (19,2%), α-cadinol (10,8%), α-pineno (10,5%) y (E) -β-ocimeno (9,0%) estaban presentes en el aceite de flores de *E. lepta*. Por otro lado, α-pineno (8,3%), trans-α-bergamoteno (7,5%), (E) -β-ocimeno (7,0%) y (E) -nerolidol (6,6%) fueron los principales constituyentes del aceite de las hojas de *E. calophylla*. Los compuestos cuantitativamente significativos del aceite de los tallos fueron (E, E)-farneseno -α (11,9%), α-terpinoleno (11,3%) y α-pineno (8,2%), mientras que α-pineno (21,6%), limoneno (19,0%) y sabineno (15,5%) se obtuvieron del aceite de las flores.

Palabras clave: *Euodia lepta*, *Euodia callophylla*, aceite esencial, monoterpenos, sesquiterpenos

Recibido | Received: February 7, 2014.
Aceptado | Accepted: October 20, 2014
Aceptado en versión corregida | Accepted in revised form: November 23, 2014
Publicado en línea | Published online: January 30, 2015
Este artículo puede ser citado como / This article must be cited as: TD Thang, TM Hoi, DN Dai, IA Ogunwande. 2015. Composition of essential oils from *Euodia lepta* (Spreng.) Merr and *Euodia calophylla* Guill., grown in Vietnam. **Bol Latinoam Caribe Plant Med Aromat 14(1): 60 – 66.**

Abbreviation List: v/w- volume by weight; GC- Gas chromatography; GC-MS- Gas chromatography coupled to mass spectrometry.

INTRODUCTION

Vietnam is a country blessed with many medicinal plants. Literature information has shown that the essential oils of these plants have received little chemical analysis. In continuation of our study on the phytochemical analysis of Vietnamese flora (Dai *et al*., 2014; Thanh *et al.*, 2014), we report herein the constituents identified in the essential oils of two species of Rutaceae collected from Nghean Province, Vietnam. *Euodia* is sometimes misspelled as *Evodia* (Hartley, 2001). In Vietnam the genus *Euodia* includes six species namely *Euodia crassifolia* Merr., *Euodia callophylla* Guillaum., *Euodia lepta* (Spreng.) Merr., *Euodia trichotoma* (Lour.) Pierre, *Euodia maeliifolia* (Hance ex Walp.) Benth and *Euodia lunu-ankenda* (Gaertn) Merr.

Euodia lepta is a perennial woody deciduous tree with hermaphrodite flowers. In ethnomedicine, the roots and leaves of *E. lepta* are used as for the treatment of various diseases such as arthritis, fever, chickenpox, fever, epidemic influenza, meningitis and infective hepatitis (Duke & Ayensu, 1985). The methanol extract of *E. lepta* was reported to displayed Syk/Src-targeted anti-inflammatory activity (Yoon *et al*., 2013), while the water extract displayed anti-oxidative activity (Bi *et al*., 2007). Phytochemical investigations of the plant resulted in the isolation and identification of several biologically active compounds including leptol A (Li *et al*., 2003), chromenes (Li *et al*., 1997a; Li *et al*., 1997b; Li *et al*., 1998a), chromans (Li *et al*., 1998b) and benzopyrane derivatives (Thang *et al*., 2007). The plant contained fatty and amino acids (Xu *et al*., 2005). A previous study (Thang *et al*., 2007) revealed that the main constituents of the volatile oil of *E. lepta* were limonene (27.22%), α-pinene (16.00%), β-pinene (10.34%) and linalool (9.18%). On the other hand, *E. callophylla* is a deciduous tree which was used in ethnomedicine for the treatment of wounds and inflammation. The main constituents of the volatile oil of this plant (Dũng *et al*., 2009) were identified to be α-pinene (9.2%), (*Z*)-β-ocimene (17.5%) and (*E*)-β-ocimene (46.6%).

The volatile compounds of some species in the genus have been reported. The essential oil extracted from the leaves of *Euodia hyladii* and *Euodia pubiflia* of Australia origin contained spathulenol as the major compound (Brophy *et al*., 2004). However, menthofuran (64%) and evodone (27%) were reported to be the major constituents of the leaves oil of *Euodia hortensis* forma *hortensis* (Brophy *et al*., 1985). The leaves oil of *Euodia trichotoma* from Vietnam (Thang *et al*., 2006) was rich in *cis*-β-ocimene (18.7%) and *trans*-β-ocimene (48.1%), while the main constituents of the flowers oil of *Euodia lunu-ankenda* from India (Sabulal *et al*., 2006) were evodione (38.9%), (*E*)-β-ocimene (12.4%), isolycodolin (11.7%) and alloevodionol (10.6%).

MATERIALS AND METHODS

Plants collection

Samples of *E. lepta* and *E. callophylla* were collected from Nghệan Province (19°20′N 104°50′E), Vietnam, in August 2012. Voucher specimens HDT 315 and HDT 304, respectively were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

Isolation of the essential oils

500 g of air-dried and pulverized samples of each plant were subjected to separate hydrodistillation for 3h at normal pressure, according to the standard procedure (Vietnamese Pharmacopoeia, 1997). The plant samples afforded lower yields of oils viz: 0.20%, 0.25% and 0.20% (v/w, *E. lepta*; leaves, stems and flowers respectively) and 0.25%, 0.32% and 0.35% (v/w; respectively for *E. callophylla* leaves, stems and flowers), calculated on a dry weight basis. All the oil samples were light yellow coloured.

Gas chromatography (GC)

GC analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS columns (30 m x 0.25 mm i.d., film thickness 0.25 μm, Agilent Technology. Berkshire, United Kingdom). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV, programmed temperature vaporisation) 250 ºC, detector temperature 260 ºC, column temperature programmed from 40 ºC (2 min hold) to 220 ºC (10 min hold) at 4 ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa.

Gas chromatographic-Mass spectrometry (GC-MS)

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm i.d., film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD (ion trap mass detector) was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas and transfer line temperature 260 ºC. 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.

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 08 Libraries (on ChemStation HP) and Wiley 9th Version and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values (Joulain & König, 1998; Adams, 2007). The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

Table 1
Chemical composition of the essential oils of *Euodia lepta* and *Euodia calophylla* grown in Vietnam

Compounds[a]	RI[b]	RI[c]	1	2	3	4	5	6
α-Thujene	930	921	1.0					0.6
α-Pinene	939	932	9.8	6.7	10.5	8.3	8.2	21.6
Camphene	954	946	0.1		0.1	1.5	0.1	0.2
cis-Carane	975	975			19.2	0.5		0.1
Sabinene	976	969				0.1	0.3	15.5
β-Pinene	980	974	0.6	1.0	0.6	0.4	0.3	
β-Myrcene	990	988	1.4	1.1	0.9	0.6	1.1	0.6
3-Hexen-1-ol-acetate	1005	1011	0.4					0.1
α-Phellandrene	1006	1002		2.1		0.1	2.5	
δ-3-Carene	1011	1006				0.1	0.2	0.1
α-Terpinene	1017	1014	0.1	0.2		Tr	0.3	0.1
p-Cymene	1024	1022				3.1	1.1	
Limonene	1032	1024	3.0	2.6	2.9	6.3	3.6	19.0
(*Z*)-β-Ocimene	1043	1032	6.3	1.0	0.8	0.6	0.5	0.5
(*E*)-β-Ocimene	1052	1044	24.4	9.9	9.0	7.0	4.4	6.1
γ-Terpinene	1061	1054	Tr			0.1	0.2	0.3
α-Terpinolene	1090	1084	Tr	4.2	0.1	0.7	11.3	
Linalool	1100	1095	1.1	0.6	1.2	0.4	0.3	0.5
6-Methyl-3,5-heptadien-2-one	1104	1106	0.2		0.5			0.5
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	1110	1105	0.2		0.1		0.1	
allo-Ocimene	1128	1128	3.7	0.7	0.5	0.5	0.5	0.4
Menthone	1142	1142	0.7	0.3	4.2			
p-Mentha-1,5-diene-8-ol	1170	1166				0.2		
Terpinen-4-ol	1177	1174				0.1		0.8
α-Terpineol	1189	1186			0.2	1.0		0.6
Decanal	1200	1200	0.2	0.4		0.2	1.0	
Verbenone	1205	1204			0.2	0.1		0.2
(*E*,*E*)-2,6-Dimethyl-3,5,7-octatriene-2-ol	1210	1207	0.6	0.3	0.4	0.2		
trans-Carveol	1217	1215			0.1	0.1		0.2

Neral	**1235**	**1235**			**0.3**	**0.4**		
Piperitone	**1268**	**1268**			**0.2**	**0.3**		
***p*-Cymen-7-ol**	**1290**	**1291**					**0.1**	**0.1**
2-Undecanone	**1291**	**1291**				**0.2**	**0.2**	
Menthyl acetate	**1297**	**1294**	**1.6**		**0.1**			
Carvacrol	**1300**	**1298**				**0.2**		
Anethole	**1301**	**1301**		**0.5**				**0.2**
Dihydrocarveyl acetate	**1305**	**1304**	**0.5**	**0.4**	**3.1**			
α-Longipinene	**1330**	**1330**					**1.9**	**1.5**
δ-Elemene	**1340**	**1335**				**0.4**	**1.1**	
α-Cubebene	**1351**	**1345**	**2.3**		**0.2**	**0.1**	**0.1**	**1.7**
(*E*)-Cyclododecene	**1356**	**1356**				**1.7**	**0.9**	
α-Ylangene	**1375**	**1373**	**0.2**			**1.9**	**0.1**	
α-Copaene	**1377**	**1374**	**0.9**	**0.4**	**0.7**	**0.4**	**0.3**	**0.4**
β-Bourbonene	**1385**	**1387**	**0.2**	**0.2**	**0.7**	**0.3**	**0.2**	
β-Cubebene	**1388**	**1387**	**0.3**				**0.3**	
β-Elemene	**1391**	**1389**	**0.3**	**0.3**	**0.3**	**0.6**	**0.7**	
α-Gurjunene	**1412**	**1409**				**0.2**	**0.4**	
β-Caryophyllene	**1419**	**1417**	**4.4**	**1.8**	**1.9**	**1.9**	**5.5**	**1.5**
***trans*-α-Bergamotene**	**1435**	**1432**				**7.5**	**0.8**	**1.8**
γ-Elemene	**1437**	**1435**				**0.9**	**0.9**	**0.5**
Aromadendrene	**1441**	**1439**				**0.2**		**0.1**
α-Humulene	**1454**	**1452**	**2.1**	**1.0**		**0.6**	**1.6**	**0.7**
γ-Gurjunene	**1477**	**1475**			**0.4**			**0.1**
Germacrene D	**1485**	**1484**		**0.6**		**0.2**		**0.4**
α-Amorphene	**1485**	**1483**	**1.4**		**1.8**			**0.4**
β-Selinene	**1486**	**1489**	**0.4**		**1.2**			**0.4**
Eudesma-4,11-diene	**1490**	**1492**		**0.8**	**0.9**			
***o*-Menth-8-ene**	**1492**	**1494**	**1.1**			**0.3**		
***cis*-Cadina-1,4-diene**	**1496**	**1495**	**0.2**	**0.5**	**1.7**			
Valencene	**1497**	**1496**	**0.2**			**0.4**	**0.2**	**0.5**
α-Selinene	**1498**	**1498**	**1.1**			**2.1**		**1.6**
α-Muurolene	**1500**	**1500**	**0.2**		**0.1**			
Lepidozene	**1502**	**1502**	**1.2**			**1.7**		
β-Bisabolene	**1506**	**1506**		**1.1**		**3.0**		
(*E*,*E*)-α-Farnesene	**1508**	**1505**	**0.3**		**0.1**	**1.0**	**11.9**	**0.2**
α-Bulnesene	**1510**	**1508**					**1.2**	
2,4-bis(1,1-Dimethyethyl)-phenol	**1513**	**1513**		**2.1**				**0.8**
γ-Cadinene	**1514**	**1513**	**1.2**			**0.4**	**0.3**	**0.4**
***cis*-α-Bisabolene epoxide**	**1515**	**1515**			**0.2**	**0.5**		**0.3**
δ-Cadinene	**1515**	**1522**	**5.2**	**1.2**	**1.3**	**2.9**	**0.9**	**0.7**
(*Z*)-γ-Bisabolene	**1531**	**1531**					**0.3**	
α-Calacorene	**1546**	**1544**	**0.1**		**0.2**	**0.1**	**0.1**	**0.5**
Elemol	**1550**	**1548**					**0.1**	
Germacrene B	**1561**	**1559**				**0.4**	**0.4**	**2.0**
(*E*)-Nerolidol	**1563**	**1561**	**3.6**	**0.1**	**1.4**	**6.6**	**1.6**	**0.8**
(Z)-3-Hexen-1-ol, benzoate	**1568**	**1568**	**0.5**		**0.4**	**0.4**	**0.1**	**0.1**
Spathulenol	**1578**	**1577**		**26.0**	**1.0**	**6.0**		**1.0**

Globulol	**1580**	**1578**	**0.3**					
Caryophyllene oxide	**1583**	**1582**	**3.0**	**0.9**	**6.9**	**0.9**	**0.6**	**1.5**
Viridiflorol	**1593**	**1592**			**0.7**	**0.1**		
Isospathulenol	**1623**	**1639**				**1.3**		
τ-Muurolol	**1646**	**1640**					**1.3**	
α-Cadinol	**1654**	**1652**	**1.2**	**1.5**	**10.8**	**2.2**	**0.6**	**0.9**
Vulgarol B	**1668**	**1668**	**0.4**			**1.3**		
Cyclotetradecane	**1669**	**1673**				**0.1**		
α-Bisabolol	**1685**	**1685**	**0.2**	**0.5**				
Juniper camphor	**1691**	**1690**	**0.1**					
Valerenol	**1715**	**1711**				**0.2**		
Farnesol[d]	**1718**		**0.3**		**0.4**	**2.2**	**1.5**	**0.3**
Mint sulfide	**1741**	**1740**					**0.4**	
Benzyl benzoate	**1760**	**1759**	**0.2**		**0.1**		**0.3**	
β-Costol	**1778**	**1776**			**1.1**		**1.1**	
6,10,14-Trimethyl-2-pentadecanone	**1884**	**1884**				**2.2**	**1.6**	
1,2-Benzenedicarboxylic acid	**1917**	**1917**		**2.2**				
***n*-Hexadecanoic acid**	**1970**	**1959**	**0.8**	**2.0**			**1.1**	**1.2**
Eicosane	**2000**	**2000**		**0.4**				
Geranyl linalool isomer	**2004**	**2002**				**2.6**	**0.8**	
Phytol	**2125**	**1942**	**0.1**	**0.5**	**0.8**			**0.1**
Octadecanoic acid	**2188**	**2170**		**0.8**		**1.0**		
Docosane	**2200**	**2200**		**0.3**				
(*Z*)-9-Octadecenamide	**2398**	**2398**		**7.7**			**1.9**	
Hexestrol	**2402**	**2402**				**4.0**	**4.6**	
Total			**90.1**	**91.1**	**91.4**	**91.9**	**90.7**	**99.9**
Monoterpene hydrocarbons			**50.4**	**31.3**	**44.5**	**29,9**	**34.6**	**65.1**
Oxygenated monoterpenes			**4.1**	**1.8**	**10.6**	**2.8**	**0.4**	**2.6**
Sesquiterpene hydrocarbons			**28.1**	**7.8**	**11.5**	**25.3**	**29.6**	**25.1**
Oxygenated sesquiterpenes			**14.3**	**29.0**	**22.5**	**26.1**	**9.2**	**4.3**
Diterpenes			**0.1**	**0.5**	**0.8**			**0.1**
Fatty acids			**0.8**	**3.5**		**2.8**	**2.0**	**1.2**
Others			**2.3**	**16.7**	**1.5**	**5.0**	**14.9**	**1.5**

[a] Elution order on HP-5MS capillary column; [b] Retention indices on HP-5MS capillary column; [c] Literature Retention indices (see Experimental); [d] Tentative assignment; Tr, trace amounts < 0.1%; 1 = *Euodia lepta* (leaves); 2 = *Euodia lepta* (stems); 3 = *Euodia lepta* (flowers); 4 = *Euodia callophylla* (leaves); 5 = *Euodia callophylla* (stems); 6 = *Euodia callophylla* (flowers)

RESULTS AND DISCUSSION

Table 1 indicates the identities and percentage compositions of the chemical constituents of the studied oil samples. The main chemical classes of compounds present in *E. lepta* leaves, stems and flowers oils are monoterpene hydrocarbons (31.3%-50.4%), and sesquiterpene hydrocarbons (7.8% - 28.1%) and oxygenated sesquiterpenes (14.3%-29.0%). The main constituents of the leaves oil were were (E)-β-ocimene (24.4%), α-pinene (9.8%), (Z)-β-ocimene (6.3%) and δ-cadinene (5.2%). The major constituents of the stems oil were spathulenol (26.0%), (E)-β-ocimene (9.9%), (Z)-9-octadecenamide (7.7%) and α-pinene (6.7%).

However, cis-carane (19.2%), α-cadinol (10.8%), α-pinene (10.5%), (E)-β-ocimene (9.0%) and caryophyllene oxide (6.9%) were the significant compounds of the flowers oil. The percentages of δ-cadinene and hexadecanoic β-pinene, limonene and linalool in the present oil samples of *E. lepta* were low when compared with previous reports (Thang *et al.*, 2006; Liang & Guo, 2009).On the other hand, monoterpene hydrocarbons (29.9%-65.1%), and sesquiterpene hydrocarbons (25.1% -29.6%) and oxygenated sesquiterpenes (4.3%-26.1%) were the main chemical classes of compounds found in *E. callophylla*. α-Pinene (8.3%), *trans*-α-bergamotene (7.9%), (*E*)-β-ocimene (7.0%), (*E*)-nerolidol (6.6%) and spathulenol (6.0%) were the major constituents present in the leaves oil, while the stems oil had its major compounds to be (*E,E*)-α-farnesene (11.9%), α-terpinolene (11.3%), α-pinene (8.2%) and (*Z*)-13-docosenamide (6.9%). The flowers gave oil whose main constituents were dominated by α-pinene (21.6%), limonene (19.0%), sabinene (15.5%) and (*E*)-β-ocimene (6.1%). The α-pinene content of the studied oil samples compared favourably with those found in a previous study (Dũng *et al*., 2009). However, the present studied oil samples of *E. callophylla* contained lower amounts of (*E*)-β-ocimene and (*Z*)-β-ocimene when compared with previous studies. In addition, sabinene, limonene, α-terpinolene and *trans*-α-bergamotene were identified in higher percentages in the present oil samples of *E. callophylla* than in previou studies.

It was noted that some compounds such as menthofuran, evodone, evodione, isolycodolin and alloevodionol that are characteristics of other *Euodia* species (Brophy *et al*., 1985; Sabulal *et al*., 2006; Thang *et al*., 2006; Dũng *et al.*, 2009) were conspicuously absent in the studied oils of *E. lepta* and *E. callophylla*.

CONCLUSION

The compositions of the leaves, stems and flowers oils of *E. lepta* and *E. callophylla* from Vietnam were reported. It was observed that the compositional patterns of the studied essential oils were different from the previously reported data. It was well known that each plant parts contained different phytochemical composition. The variation between these results and those from other parts of the world may be due to the ecological and climatic differences between these regions; as well as the age of the plants and chemotype.

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