Artículo original

Study of the chemical composition of the essential oil from the hybrid Asteraceae *Carramboa tachirensis* (Aristeg.) Cuatrec.

Estudio de la composición química del aceite esencial de la Asteraceae hibrida *Carramboa tachirensis* (Aristeg.) Cuatrec.

Obregón-Díaz Ysbelia[1], Rojas-Fermín Luis[1*], Usubillaga Alfredo[1], Pouységu Laurent[2], Quideau Stéphane[2].

[1] Instituto de Investigaciones, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida C.P. 5101. República Bolivariana de Venezuela. [2]Université de Bordeaux, Institut des Sciences Moléculaires (CNRS-UMR 5255) and Institut Européen de Chimie et Biologie, 2 rue Robert Escarpit, 33607 Pessac Cedex, France.

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RESUMEN

Carramboa tachirensis (Aristeg.) Cuatrec. es un híbrido perteneciente a la subtribu Espeletiinae (Asteraceae), que crece en el páramo El Batallón a una altitud de 2800 m y cuyas especies parentales son *Carramboa pittieri* (Cuatrec.) Cuatrec. y *Ruilopezia marcescens* (S.F. Blake) Cuatrec. El aceite esencial de las hojas de esta planta fue aislado por hidrodestilación y su composición química fue determinada por cromatografía de gases–detector de ionización de llama y cromatografía de gases–espectrometría de masas. Se identificaron 38 componentes por comparación de sus espectros de masas con los datos de las librerías Wiley y NIST. Los componentes mayoritarios fueron germacreno-D (44,4 %), *trans*-β-Guaieno (8,0 %), E-γ-bisaboleno (3,2 %), β-cariofileno (3,0 %), α-pineno (2,3 %), α-copaeno (2,2 %) y *ent*-kaur-16-eno-19-al (6,6 %). El aceite de este híbrido presenta mayor similitud en su composición a la del aceite de *C. pittieri* (Cuatrec.) Cuatrec. que al aceite de *R. marcescens* (S.F. Blake) Cuatrec.

PALABRAS CLAVE

Carramboa tachirensis, Asteraceae, composición aceite esencial, germacreno-D.

ABSTRACT

Carramboa tachirensis (Aristeg.) Cuatrec. is a hybrid species that belongs to the subtribe Espeletiinae, a member of the Asteraceae family. It grows at paramo El Batallón at an altitude of 2800 m and its parental species are *Carramboa pittieri* (Cuatrec.) Cuatrec. and *Ruilopezia marcescens* (S.F. Blake) Cuatrec. The essential oil from the leaves of this plant was isolated by hydrodistillation and its chemical composition was determined by GC-FID and GC-MS. A total of 38 components were identified by comparison of their mass spectra with Wiley and NIST Library data. The major constituents were germacrene-D (44.4 %), *trans*-β-guaiene (8.0 %), E-γ-bisabolene (3.2 %), β-caryophyllene (3.0 %), α-pinene (2.3 %), α-copaene (2.2 %) and *ent*-kaur-16-en-19-al (6.6 %). The essential oil of this hybrid looks more as the essential oil of *C. pittieri* (Cuatrec.) Cuatrec. than the oil of *R. marcescens* (S.F. Blake) Cuatrec.

KEY WORDS

Carramboa tachirensis, Asteraceae, essential oil composition, germacrene-D.

INTRODUCTION

Frailejón is the popular name given to

*Correspondencia al autor: rojasl@ula.ve

a group of resinous plants that grow above 2000 m of altitude in the Northern Andes. The genus *Espeletia "sensu lata"* was used to gather all known species of frailejón, but in 1976 Cuatrecasas [1] created the subtribe Espeletiinae and distributed these plants in seven genera: *Carramboa, Coespeletia, Espeletia, Espeletiopsis, Libanothamnus, Ruilopezia* and *Tamania,* according to their habit and other vegetative characters. A few years later Cuatrecasas created an additional genus *Paramiflos* [2]. The Espeletiinae is the most representative taxonomic group of the paramo plant life. Most of them have a characteristic rosette form, but *Carramboa* species are different, since they are small trees about 10 m tall that grow on the transition zone between the lower paramo and the tropical woods (2000-2500 m of altitude). There are 105 species of Espeletiinae in Colombia, 75 in Venezuela, and one in Ecuador, but *Carramboa* are only found in Venezuela and thus far only six taxa have been accepted: *Carramboa badilloi, Carramboa pittieri* (Cuatrec.) Cuatrec., *Carramboa trujillensis* (Cuatrec.) Cuatrec., *Carramboa rodriguesii* (Cuatrec.) Cuatrec. *Carramboa wurdackii* (Ruiz-Teran & López-Fig.) Cuatrec. and *Carramboa tachirensis* (Aristeg.) Cuatrec. which is an hybrid of *Carramboa pittieri* (Cuatrec.) Cuatrec. and *Ruilopezia marcescens* (S.F. Blake) Cuatrec. This genus, regarded is a primitive taxum and has been described by Morillo and Briceño [3,4]. Hybridization is frequent in Espeletiinae, and since all taxa thus far studied have 19 chromosomes [5,6] which, added to the fact that *C. pittieri* (Cuatrec.) Cuatrec. and *R. marcescens* (S.F. Blake) Cuatrec. grow in the same area, makes such hybridization possible. In previous reports the oil of *C. pittieri* Aristeg. contains the same components as that of *C. tachirensis*, but in different proportions.

MATERIAL AND METHODS

Plant material. Leaves of *C. tachirensis* (Aristeg.) Cuatrec., were collected in Paramo Batallón on the way to Pregonero, at 2800 m of altitude. A Voucher specimen (G. Morillo y L. Rojas 13520) was deposited at the MERF Herbarium and identified by Professor Gilberto Morillo, Faculty of Forestry and Environmental Sciences of the University of Los Andes.

Extraction of the essential oil. The fresh leaves (1000 g) were cut into small pieces and the volatile fraction was isolated by hydrodistillation during 2 h using a Clevenger trap. The oil was dried over anhydrous sodium sulfate and stored at 4 °C.

Gas Chromatography. GC analyses were performed using a Perkin-Elmer Autosystem gas chromatograph equipped with a FID detector and data-handling system. A 5% phenylmethylpolysiloxane fused-silica capillary column was used (30 m x 0.25 mm i.d., film thickness 0.25 μm; HP-5, Hewlett-Packard, CA, USA). The oven temperature was programmed from 60 ºC to 260 ºC at 4 ºC/min. The injector and detector (FID) temperatures were 200 ºC and 280 ºC, respectively. The carrier gas was helium at 0.8 mL/min. The sample (1.0 μL) was injected using a split ratio of 10:1. Retention indices were calculated by comparing the retention times of the eluting peaks with those of standard C_8-C_{24} *n*-alkanes. The percentage composition of the oil was calculated by the normalization method from the GC peak areas.

Gas Chromatography – Mass Spectrometry. GC-MS analyses were carried out on a Model 5973 Hewlett-Packard GC-MS system fitted with a HP-5MS fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm, Hewlett-Packard). The oven temperature program was the same as that used for the GC-FID analysis; the transfer line temperature was programmed from 150 ºC to 280 ºC; the source temperature was 230 ºC; and the quadrupole temperature, 150 ºC. The carrier gas, was helium, adjusted to a linear velocity of 34 cm/s; ionization energy, 70 eV; scan range, 40:500 amu; 3.9 scans/s. The sample (1.0 μL) was injected using a Hewlett-Packard ALS injector with a split ratio of 50:1. The identity of the oil components was established from their GC retention indices, by comparison of their MS spectra with those of standard compounds available in the laboratory, and by a library search (NIST 05 and Wiley) [7].

TABLE 1

Percentage composition of the essential oils of the species: *Carramboa tachirensis* (Aristeg.) Cuatrec., *Carramboa pittieri* (Cuatrec.) Cuatrec. and *Ruilopezia marcescens* (S.F. Blake) Cuatrec.

N°	Compounds	*C. tachirensis*		*C. pittieri* *	*R. marcescens*** Jun 1999	*R. marcescens**** Jan 2000
		RI	Area %	Area %	Area %	Area %
1	*cis*-3-hexen-ol	-	-	0.1	-	-
2	*α*-pinene	945	2.3	1.7	25.0	32.9
3	camphene	945	-	-	< 0.1	0.2
4	sabinene	963	-	0.1	0.5	1.1
5	*β*-pinene	986	1.4	0.4	6.4	2.3
6	*β*-myrcene	997	0.8	4.4	0.5	0.4
7	*α*-phellandrene	1013	0.6	1.3	8.4	12.3
8	*p*-cymene	1034	0.3	0.8	5.4	9.2
9	limonene	1039	0.3	0.2	1.2	1.5
10	*γ*-terpinene	1055	-	-	1.2	0.7
11	*cis*-sabinene hydrate	1062	-	-	< 0.1	0.8
12	*α*-terpinolene	1079	-	-	< 0.1	0.3
13	linalool	1110	0.2	-	0.4	< 0.1
14	*p*-mentha-1,5-diene-8-ol	1161	-	-	< 0.1	0.3
15	terpin-4-ol	1171	-	-	2.0	1.6
16	*α*-terpineol	1183	-	-	2.1	1.5
17	myrtenal	1190	-	-	0.7	0.5
18	myrtenol	1206	0.3	-	-	-
19	cyclosativene	1379	0.3	0.8	-	-
20	*α*-copaene	1378	2.2	5.1	-	-
21	*β*-bourbenene	1386	-	0.1	-	-
22	*β*-cubebene	1396	0.2	0.3	-	-
23	*trans*-caryophyllene	1410	-	-	1.6	0.7
24	*α-cis*-bergamotene	1425	0.4	-	-	-
25	*β*-caryophyllene	1431	3.0	6.1	-	-
26	*trans-α*-bergamotene	1448	1.1	-	-	-
27	6,9-guaiadiene	1456	0.9	-	-	-
28	*α*-humulene	1468	0.9	0.9	0.4	0.4
29	alloaromandrene	1463	-	0.5	-	-
30	*λ*-gurjunene	1472	-	0.2	-	-
31	*λ*-muurolene	1481	-	0.7	-	-
32	*β*-selinene	1492	0.7	8.2	-	-
33	*epi*-cubebol	1498	-	0.5	-	-
34	germacrene-D	1501	44.4	28.9	33.5	24.9
35	valencene	1504	0.2	0.3	0.9	0.4
36	*δ*-selinene	1507	0.9	-	-	-
37	*α*-muurolene	1510	0.6	0.5	-	-
38	λ-bisabolone	1511	-	0.5	-	-
39	bicyclogermacrene	1513	2.1	1.2	2.8	1.5
40	*trans-β*-guaiene	1523	8.0	-	-	-
41	*γ*-cadinene	1530	0.6	-	-	-
42	*δ*-cadinene	1537	1.3	2.9	-	-
43	E-*γ*-bisabolene	1545	3.2	-	1.0	0.9
44	germacrene-B	1568	0.4	-	-	-
45	*trans*-nerolidol	1566	-	0.6	-	-
46	nerolidol-E	1572	1.0	-	-	-
47	1,6-germacradien-5-ol	1578	-	0.2	-	-
48	spathulenol	1580	-	0.9	0.8	0.5
49	caryophyllene oxide	1591	0.6	1.2	-	-
50	guaiol	1603	-	0.7	-	-
51	humulene 1,2-epoxide	1606	-	0.2	-	-
52	1,10-di-*epi*-cubenol	1616	-	0.2	-	-
53	*epi-α*-muurolol	1623	-	0.8	-	-
54	cubenol	1625	-	0.4	-	-
55	cedr-8(15)-en-9-*α*-ol	1627	-	0.8	-	-
56	10-*epi-γ*-eudesmol	1631	0.5	-	-	-

TABLE 1. Continued
Percentage composition of the essential oils of the species: *Carramboa tachirensis* (Aristeg.) Cuatrec., *Carramboa pittieri* (Cuatrec.) Cuatrec. and *Ruilopezia marcescens* (S.F. Blake) Cuatrec.

N°	Compounds	*C. tachirensis*		*C. pittieri* *	*R. marcescens*** Jun 1999	*R. marcescens**** Jan 2000
		RI	Area %	Area %	Area %	Area %
57	*α*-bisabolol	1644	-	3.5	-	-
58	*α*-cadinol	1670	1.1	-	-	-
59	18-nor-*ent*-16-kaurene	1969	1.1	-	-	-
60	19-nor-*ent*-16-kaurene	2005	1.0	-	-	-
61	manool oxide	2030	0.4	0.3	-	-
62	*ent*-kaurene	2064	1.2	1.2	-	-
63	epi-ruilopeziol	2195	0.2	-	-	-
64	kauran-16-ol	2259	1.0	1.2	-	-
65	*ent*-kaur-16-en-19-al	2292	6.6	15.1	0.5	0.3
66	19-hydroxy-*ent*-kaur-16-ene	2360	-	0.2	-	-
Identified components (%)			**92.3**	**94.2**	**95.3**	**95.2**
Monoterpene (%)			**6.2**	**8.9**	**53.8**	**65.6**
Sesquiterpene (%)			**74.6**	**67.2**	**41**	**26.3**
Diterpene (%)			**11.4**	**17.5**	**0.5**	**0.3**

RI: retention index, t: < 0.1; * Rojas *et al*., 2008; ** Aparicio *et al.*, 2001 and *** Aparicio *et al*., 2001.

RESULTS AND DISCUSSION

The retention indices and percentage chemical composition of the essential oil of *C. tachirensis* (Aristeg.) Cuatrec. is listed on Table 1 in order of elution from an HP-5 capillary column. The identified compounds represent 92.2 % of the oil. The major constituents were germacrene D (44.4 %), *trans*-*β*-guaiene (8.0 %), E-*γ*-bisabolene (3.2 %), *β*-caryophyllene (3.0 %), *α*-pinene (2.3 %), *α*-copaene (2.2 %), and *ent*-kaur-16-en-19-al (6.6 %). Previous studies on the oil of *C. pittieri* (Cuatrec.) Cuatrec. [8] and *Carramboa littlei* Aristeg. [9] showed that in these two species germacrene-D and *ent*-kaur-16-en-19-al were also major constituents. On the other hand it is worth mentioning that *C. tachirensis* (Aristeg.) Cuatrec. contains 18-nor-*ent*-16-kaurene (1.1 %) and 19-nor-*ent*-16-kaurene (1.0 %) two nor-kaurenes isolated by Bohlmann from *Libanothamnus granatesianus* (Cuatrec.) Cuatrec. [10].

As can be observed on Table 1, monoterpenes constitute more than 50 % of the oil in *R. marcescens* (S.F. Blake) Cuatrec. (53.8 % in June and 65.6 in January) [11] while they made up only 8.9 % and 6.2 % in the oils of *C. pittieri* (Cuatrec.) Cuatrec. and the hybrid respectively. On the other hand sesquiterpenes are particularly abundant in the oil of *C. pittieri* (Cuatrec.) Cuatrec. (67.2 %) as well as on that of the hybrid (74.6 %), but less abundant in *R. marcescens* (S.F. Blake) Cuatrec. oil. This difference is even more noticeable with relation to diterpenes, since the oil of *C. pittieri* (Cuatrec.) Cuatrec. contains 17.5 % of diterpenes and these compounds are also abundant in the hybrids'oil (11.4 %). On the other hand *R. marcescens* (S.F. Blake) Cuatrec. oil contains 0.5 % or even less diterpenes. Accordingly, it is obvious that the composition of the hybrid's oil looks more like the composition of the oil of *C. pittieri* (Cuatrec.) Cuatrec.

CONCLUSIONS

The essential oil of *C. tachirensis* (Aristeg.) Cuatrec. was found to contain 38 volatile compounds which represent 92.2 % of the oil. Sesquiterpenes, constitute 74.6 % of the oil, while diterpenes (11.4 %) and monoterpenes (6.2 %) follow in importance. Since *C. tachirensis* (Aristeg.) Cuatrec. is a hybrid species the composition of its essential oil was found to have a composition that is an average between the oil composition of its parental species. It has been reported [12] that oils that contain germacrene-D as a major constituent possess antibacterial and anti-oxidizing activity.

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