Analysis of the acid and neutral fractions present in *Carramboa tachirensis* (Aristeg.) Cuatrec. (Asteraceae) by Gas Chromatography-Mass Spectrometry

Análisis de las fracciones ácida y neutra presentes en *Carramboa tachirensis* (Aristeg.) Cuatrec. (Asteraceae) por Cromatografía de Gases-Espectormetría de masa

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Abstract

The genus *Carramboa* (family Asteraceae) comprises seven species, among which is the *Carramboa tachirensis* (Aristeg.) (Cuatrec.), species endemic to the Andean paramos. In this paper we prepared an extract of n-hexane: diethyl ether (3:1) from dried leaves of the plant then followed a march acid-base, yielding two fractions: an acid, which was methylated, and one neutral. Both were analyzed by Gas Chromatography-Mass Spectrometry. The identification was made by comparison with authentic standards and with the use of databases Wiley (6th Edition), Nist 05, and Adams. Compounds identified were: *ent*-kaur-16-en-19-oic acid (11.16 %), *ent*-kauran-19-oic acid (9.18 %) and *ent*-15α-hydroxy-16-en-kaur-19-oic acid (17.03 %) of the acid fraction and *ent*-kaur-16-en-19-ol (2.63 %), *epi*-ruilopeziol (5.19 %) and 16β-kaurano (14.47 %) respectively the neutral fraction. These identified compounds are derivatives of *ent*-kaurene and are frequently found in species belonging to the sub-tribe Espeletiinae. This is the first phytochemical report for *Carramboa tachirensis* and the genus *Carramboa*.

Keywords: Asteraceae, *Carramboa tachirensis*, kaurenics acids, Gas Chromatography-Mass Spectrometry

Resumen

El género *Carramboa* (familia Asteraceae) comprende siete especies, dentro de las cuales se encuentra la *Carramboa tachirensis* (Aristeg.) (Cuatrec.), especie endémica de los páramos andinos. En el presente trabajo se preparó un extracto de n-hexano:éter dietílico (3:1) a partir de hojas secas de la planta, posteriormente se siguió una marcha ácido-base, obteniéndose dos fracciones: una ácida, la cual fue metilada, y otra neutra. Ambas fueron analizadas por Cromatografía de Gases-Espectormetría de masa. La identificación se realizó mediante el empleo de las bases de datos Wiley (6ta Edición), Nist 05 y Adams. Los compuestos detectados fueron: ácido *ent* kaur-16-eno-19-oico (11,16 %), ácido *ent*-kaurano-19-oico (9,18%) y ácido *ent*-15α-hidroxi-16-eno-kaur-19-oico (17,03 %) de la fracción ácida y también *ent*-kaur-16-eno-19-ol (2,63 %), *epi*-ruilopeziol (5,19 %) y 16β-kaurano (14,47 %) de la fracción neutra. Estos compuestos identificados son derivados del *ent*-kaureno siendo encontrados en especies pertenecientes a la sub-tribu Espeletiinae. Este es el primer reporte fitoquímico para *Carramboa tachirensis* y del género *Carramboa*.

Palabras clave: Asteraceae, *Carramboa tachirensis* , ácidos kaurénicos, Cromatografía de Gases-Espectormetría de masa.

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Introduction

Asteraceae or Compositae belong to the subclass Asteridae, Asterales order, which contains only this family, according to the classification system of Cronquist (1981) (Badillo, 1997). More recently, APG (2003) keeps Asteraceae in order Asterales but accompanied by ten families. It is one of the dominant taxa in the flora of Venezuela and consists of herbaceous species, shrubs, and climbers. The number of species increases progressively in the tropics, especially in high-altitude mountain sites and few representatives in lowlands forest formations (Nagashima et al., 2003). According to Llamozas et al. (2003), represents the family Asteraceae taxon angiosperm third highest number of species in Venezuela (805). All these species are included in 13 tribes and the largest in the Heliantheae, which includes herbs, shrubs, and small trees in a total of 72 genera (Badillo, 1997). Some of the unique genus of high Andean mountains and moors are *Carramboa, Coespeletia, Espeletia, Espeletiopsis, Libanothamnus, Ruilopezia, Paramiflos* and *Tamania.* The genus *Carramboa* (family Asteraceae), formerly belong to *Espeletias*, was created by Cuatrecasas (1976) and comprises 7 species, among which is the *Carramboa tachirensis* (Aristeg.) Cuatrec. (Aristeguieta, 2003), endemic to the Andean highlands and outside the sub-tribe Espeletiinae, this genus, regarded as a relatively primitive, usually unbranched trunk has alternate leaves clustered at the ends of branches and flowers in various shades of yellow (Cuatrecasas, 1976, 2013). The presence of taxa within the genus with leaves of varying size and shape, and the remarkable polymorphism chapters, suggested the possibility of hybrid populations, based on studies by Morillo and Briceño in 2007, verified the taxonomics status of that *C. tachirensis*, which is a hybrid taxon between *Carramboa pittieri* (Cuatrec.) Cuatrec. and *Ruilopezia marcescens* (Blake) Cuatrec. In the study of the essential oil the leaves the *Carramboa tachirensis* (Aristeg.) Cuatrec., a total of 38 components were identified, with the main components germacreno-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 %) (Obregón-Díaz et al., 2015). The volatile components of parental species, *Carramboa pittieri* were analyzed and were detected some monoterpenes, sesquiterpenes, and the *ent*-kaur-16-en-19-al and *ent*-kaur-16-en-19-ol (Rojas et al., 2008). The oil of this *C. tachirensis* hybrid has a greater similarity in its composition to that of oil *C. pittieri* (Cuatrec.) Cuatrec. It is worth mentioning that phytochemical studies of other genera belonging to the Espeletiinae subtribe have been reported (Peña et al., 2012; Aparicio et al., 2013; Morillo et al., 2017). Various biological activities have been described for *ent*-kaurene diterpenes and their derivatives such as plant growth regulators, antimicrobial, antiparasitic and insects, cytotoxic, antitumoral, anti-HIV, androgenic, anti-fertility, as well as hypotensive activity, analgesic, and anti-inflammatory, among others (Ghisalberti, 1997; Cordero et al., 2017; Ríos et al., 2017; Cordero et al., 2021). Some of these compounds are polyhydric and most of them were found cytotoxic activity against several cancer cell lines (Bruno-Colmenarez et al., 2011). This is the first phytochemical report for *Carramboa tachirensis* and the genus *Carramboa*.

Materials 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 (Morillo y 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.

Obtaining extract n-hexane: ether

Fresh leaves of *C. tachirensis* were dried in the oven at 35 °C for 3 days; from ground dried material (689.95 g) underwent removal of n-hexane: diethyl ether (3:1) (J.T.Baker®, Riedel-de Haën®) at room temperature for 4 days. The crude extract was concentrated to dryness to give a solid residue of 34.45 g.

Isolation kaurenics acids

A 34.45 g of the crude extract was dissolved in a mixture of n-hexane-diethyl ether (3:1), stirred with a 5 % solution of NaOH (JT Baker®). The resulting aqueous phase was acidified with concentrated HCl (Riedel-de Haën®) to pH 3 and stirred with n-hexane-diethyl ether (3:1) to give 0.7395 g of the acid fraction. Moreover, the neutral fraction obtained was decolorized by boiling with activated charcoal in hexane and subsequently purified through a silica gel column to afford 8.559 g of solid material.

Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

Methylation of the acid fraction

A small portion (10 mg) of the acid reaction was subjected to methylation with diazomethane generated in situ in ethereal solution and subsequently analyzed by GC-MS using Hewlett-Packard 6890 gas chromatograph fitted with an HP 5973 mass detector was used. A 30 m long (0.25 mm d.i. and 0.25 μm film) HP-5MS capillary column was used. The ionization energy was 70 eV, the analysis mass range was 40:500 amu at 3.9 scans/s. The program using a Hewlett-Packard ALS injector, applying a 30:1 split ratio. Helium at 0.9 mL/min was used as carrier gas. The following temperature program was used: initial temperature 100 °C, which was increased at 10 °C/min to 200 °C. A second temperature increase of 8 °C/min was applied to a final temperature of 300 °C to obtain a total analysis time of 30 min. The identification was made by comparison with authentic standards and the use of databases Wiley (6th Edition), NIST 05 and Adams (2007). Furthermore, a comparison of retention times and mass spectra of each of the components with existing patterns in the Research Institute of the Faculty of Pharmacy and Bioanalysis, University of Los Andes.

Results and discussion

The GC-MS analysis of the acid fraction methylated with diazomethane allowed the identification of various kaurenics acids present in the resin *C. tachirensis*. The retention times and the relative abundance of the methyl esters of kaurenics acids identified can be seen in Table I, and their respective structures are shown in Figure 1. Kaurene type diterpenes: *ent*-kaur-9(11),16-dien-19-oic acid (grandiflorénic acid, 4) *ent*-kaur-16-en-19-oic acid (kaurenic acid, 5), *ent*-kauran-16-hydroxy-19-oic acid (6), *ent*-kauran-19-oic acid (7), *ent*-kaur-15α-hydroxy-16-en-19-oic acid (grandiflorólic acid, 8), *ent*-kaur-15-oxo-16-en-19-oic acid (9), *ent*-kaur-17-

hidroxy-15-en-19-oic acid (10), *ent*-kaur-15α-*O*-acetoxy-16-en-19-oic acid (11), *ent*-kaur-15-*O*-isobutiloxy-16-en-19-oic acid (12) and *ent*-kaur-15α-isovalerioxy-16-en-19-oic acid (13) represent the main component of the acidic fraction (63.74% of the same, Table I) and all of them have been reported in several species of the genera *Espeletia, Coespeletia, Libanothamus* and *Ruilopezia* the subtribe Espeletiinae (Usubillaga and Capra, 1988; Usubillaga et al., 2001; Usubillaga et al., 2003; Meccia et al., 2010; Peña et al., 2012; Aparicio et al., 2013), for that reason are considered important as ethnobotanical markers in different types of frailejones.

The percentage of abundance resulting of acids found in the leaves of *C. tachirensis* was kaurenic acid (11.16 %), *ent*-kauran-19-oic acid (9.18 %), and acid *ent*-15α-hydroxy-16-en-kaur-19-oic acid (17.03%). These results are consistent with those reported by Morillo et al. (2017), although in different concentrations, they are reported to be in the presence of *ent*-kaur-15α-hydroxy-16-en-19-oic acid as the main component (29.3%), as well as *ent*-kaur-16-en-19-oic acid (17.0%). For both investigations, the conditions of the chromatographic program were similar which allowed us to compare the results obtained. It is important to mention that kaurenic acid has various biological properties such as anti-inflammatory, antipyretic, antibacterial, cytotoxic, and antiparasitic (Ghisalberti, 1997; Cordero et al., 2012; Ríos et al., 2017; Cordero et al., 2021), this compound also known as grandiflorólic acid was first isolated from *Espeletia grandiflora*, an Espeletiinae of Colombia (Nagashima et al., 2003; Bruno et al., 2011). The remaining 36.26 % of this fraction corresponds to other acidic substances minority, possibly derived from *ent*-kaurene that has not been identified.

From the extract of leaves, neutral fractions were identified by GC-MS. The *ent*-kaur-16-en-19-ol (kaurenol) with an

Table I

Kaurénics acids present in the acid fraction of *C. tachirensis* analyzed as methyl esters

Nº	Compounds	Time (min.)	Area (%)
1	Hexadecanoic acid methyl ester	12.69	2.34
2	Hexadecanoic acid ethyl ester	13.42	0.98
3	Octadecanoic acid methyl ester	14.85	0.95
4	*ent*-kaur-9(11),16-dien-19-oic acid methyl ester	15.97	1.89
5	***ent*-kaur-16-en-19-oic acid methyl ester**	**16.94**	**11.16**
6	*ent*-kauran-16α-hydroxy-19-oic acid methyl ester	18.90	2.64
7	***ent*-kauran-19-oic acid methyl ester**	**19.00**	**9.18**
8	***ent*-kaur-15α-hydroxy-16-en-19-oic acid methyl ester**	**19.26**	**17.03**
9	*ent*-kaur-15-oxo-16-en-19-oic acid methyl ester	19.50	5.01
10	*ent*-kauran-17-hydroxy-15-en-19-oic acid methyl ester	19.62	3.80
11	*ent*-kaur-15α-*O*-acetoxy-16-en-19-oic acid methyl ester	20.84	0.90
12	*ent*-kaur-15α-*O*-isobutiloxy-16-en-19-oic acid methyl ester	21.15	2.27
13	*ent*-kaur-15α-isovalerioxy-16-en-19-oic acid methyl ester	22.04	5.59
Total			**63.74**

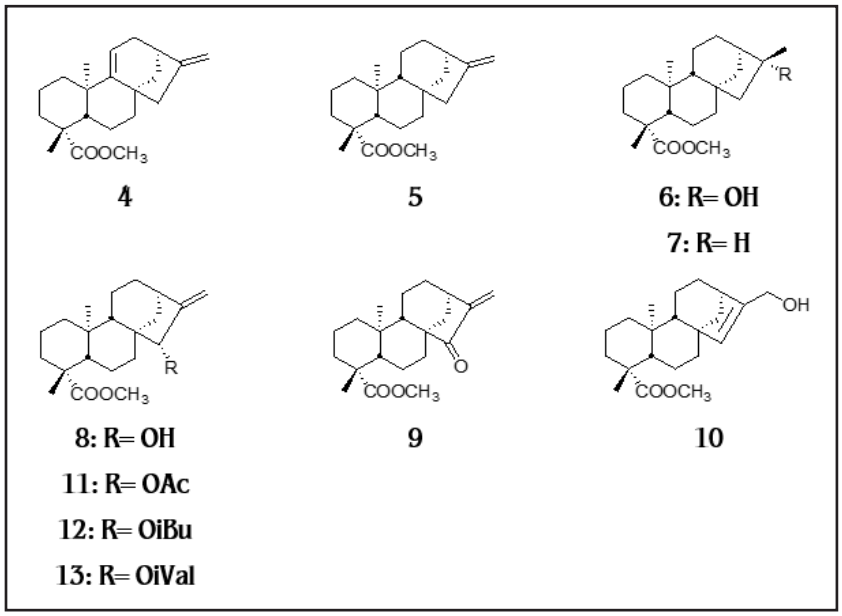

Figure 1. Structures of kaurenics acids identified in the resin acid fraction of *C. tachirensis.*

abundance of 2.63%, ruilopeziol (2.30%), *epi*-ruilopeziol (5.19%), 16β-kaurano (14.47%) and 16α-kaurane (2.78 %) respectively, (Figure 2) while 3.83 % is presumed to correspond to a small amount of diterpenes of the *ent*-kaurene series unidentified as shown in Table II. These compounds are derivatives of *ent*-kaurene and are frequently found in species belonging to the subtribe Espeletiinae (Cuatrecasas, 1976).

Regarding the compounds identified in the neutral fraction, the diterpene *ent*-kaur-16-en-19-ol (kaurenol, 8) is important because from this compound have been obtained several semisynthetic derivatives to target new active substances; as in the work of Batista et al. (2007), they modified this diterpene and get derivatives biologically active against trypomastigotes the *Trypanosoma cruzzi.* Additionally, Hueso-Falcón et al. (2011) and Ríos et al. (2017) get new derivatives from kaurene with anti-inflammatory activity.

Conclusions

This research is, to our knowledge, the first report made phytochemical to *Carramboa tachirensis* (Aristeg.) Cuatrec., and it was shown that, like many other species of the subtribe Espeletiinae (family Asteraceae), the leaves mainly produce diterpenes kaurene type.

The acidic fraction analysis allowed the identification of ten major acid series *ent*-kaur-19-oic (corresponding to 59.47% of total); additionally, in the neutral fraction was found significant percentage alcohols as 16β-kauranol, *epi*-ruilopeziol, 16α-kaurane, and *ent*-kaur-16-en-19-ol. The analysis of the methyl esters by GC-MS showed *ent*-kaur-16-en-19-oic acid (11.16%) and *ent*-kaur-15α-hydroxy-16-en-19-oic acid

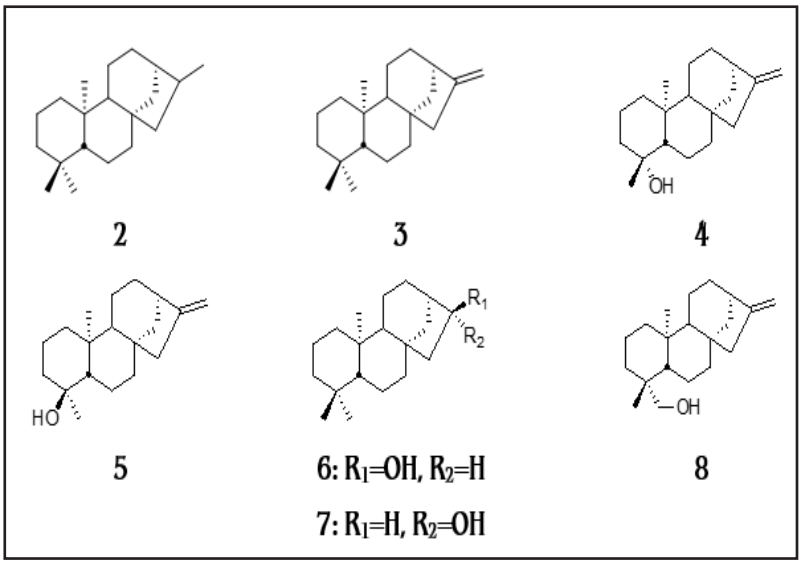

Figure 2. Structures of kaurenics identified in the extract of leaves neutral fraction of *C. tachirensis.*

Table II
Components identified in the resins neutral fraction obtained *C. tachirensis*

Nº	Compounds	Time (min.)	Area (%)
1	Pentadecanoic acid methyl ester	3.93	0.78
2	Kaurane	4.62	1.29
3	Kaur-16-ene	5.11	1.04
4	ruilopeziol	5.76	2.30
5	*epi*-ruilopeziol	6.03	5.19
6	**16β-Kauranol**	**6.44**	**14.47**
7	16α–Kauranol	6.62	2.78
8	*Ent*-kaur-16-en-19-ol	7.26	2.63
9	Pentacosano (C25)	7.77	0.90
10	**Heptacosane (C27)**	**9.20**	**30.42**
11	Octacosane (C28)	9.88	1.98
12	**Nonacosane (C29)**	**10.59**	**24.48**
13	Triacontane (C30)	11.36	1.49
14	Hentriacontane (C31)	12.27	6.42
Total			**96.17**

(17.03%) are the most abundant acids in this species, while *ent*-kaur-19-oic acid was found to be the third in abundance, with 9.18 %. Special interest has the *ent*-kaur-15α-hydroxy-16-en-19-oic acid compound, because of this acid is obtained 15-oxo-kaur-16-en-19-oic acid, a substance which induces apoptosis of epithelial cells of prostate cancer (Ruiz et al., 2008) and is generally low in most of the species examined phytochemical frailejón in our laboratory. The *ent*-15-oxo-kaur-16-en-19-oic acid was obtained first by hemisynthesis by Cannon et al. (1966) and later isolated as a natural compound *Xylopia acutiflora* species by Hasan et al. (1982), but their production is not available from natural sources (Aparicio et al., 2007, 2013).

Importantly, as shown in the results tables a percentage of approximately 36.26% in the acidic fraction and 3.83% in the neutral fraction, was not possible to identify, because there is no pattern matching on the computer. To elucidate and identify the major structures of the compounds reported, it is necessary to isolate a more significant amount of each extract and analyze these compounds by the technique of nuclear magnetic resonance spectroscopic studies, which were not possible in this investigation due to the small amount of sample collected.

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References

Adams RP. 2007. Identifications of essential oil components by Gas Chromatography-Mass Spectrometer, ed 4.1. Biology Department. Baylor University. Pp 1–809.

Aparicio RL, Bahsas AH, Usubillaga del Hierro A. 2007. Allylic oxidation of *ent*-kaurenic acid, *ent*-kaurenic acid methyl ester and *ent*-kaurenol. Avan Quím 2(3): 3–8.

Aparicio RL, Villasmil TO, Peña A, Rojas J, Usubillaga A. 2013. Estudio fitoquímico de las hojas de *Espeletia semiglobulata* Cuatrec. Rev Fac Farm 55(2): 2–5.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. Bot J Linn Soc 141: 399–436.

Aristeguieta LS. 2003. Estudio dendrológico de la flora de Venezuela. Academia de Ciencias Físicas, Matemáticas y Naturales. Vol. XXXVIII. Caracas.

Badillo V.M. 1997. Los géneros de las Compositae (Asteraceae) de Venezuela. Clave artificial para su identificación. Ernstia 6(2-3): 51–168.

Batista R., Humberto J.L, Chiari E, Braga de Oliveira

A.B. 2007. Synthesis and trypanocidal activity of *ent*-kaurane glycosides. Bioorg. Med Chem 15(1): 381–391.

Bruno-Colmenarez J, Díaz De Delgado GC, Peña A, Alarcón L.V, Usubillaga A, Delgado-Méndez P. 2011. Structure of *ent*-15α-hydroxy-kaur-16-en-19-oic acid. Avan Quím 6(2): 16–20.

Cannon J.R., Chow PW, Jeffreries PR, Meehan G.V. 1966. Isolation of (-)-Kaur-16-en-19-oic acid and 15β-Hydroxy-(–)-kaur-16-en-l9-oic acid from *Phebalium rude* Bartl. Aust J Chem 19(5): 861–867.

Cordero YE, Corao GM, Cova JA, Usubillaga A. 2012. Effect of some *ent*-kaurenes on the viability of human peripheral blood mononuclear cells. Nat Prod Commun 7: 563–564.

Cordero de Rojas Y, Ustáriz L, Araujo L, Usubillaga A, Rojas F, Moujir L. 2017. Actividad antibacteriana de diterpenos del kaurano aislados de *Coespeletia moritziana* (Sch. Bip. ex Wedd.) Cuatrec. Rev Fac Farm 59(2):03–07.

Cordero YE, Lucena M, Araujo L, Usubillaga, A, Rojas L, Aparicio R, Moujir L, Ustáriz F. 2021. Efecto citotóxico de diterpenos derivados del àcido *ent*-kaureno aislados de *Coespeletia moritziana* (Sch. Bip. ex Wedd.) Cuatrec." BLACPMA. In Press.

Cronquist A. An integrated system of classification of flowering plants. Columbia University Press, New York, 1981.

Cuatrecasas J. 1976. A new subtribe in the Heliantheae (Compositae): Espeletiinae. Phytologia 35:43–61.

Cuatrecasas J. 2013. A systematic study of the subtribe Espeletiinae: Heliantheae, Asteraceae. Memoirs of the New York Botanical Garden 107: 689.

Ghisalberti E. 1997. The biological activity of naturally occurring kaurane diterpenes. Fitoterapia 58(4): 303–325.

Hasan CH, Healey T, Waterman P. 1982. Kaurane and kaurene diterpenes from the stem bark of Xylopia acutiflora. Phytochemistry 21(8): 2134–2135.

Hueso-Falcon I, Cuadrado I, Cidre F, Amaro-Luis J.M, Ravelo A.G, Estévez-Braun A De las Heras B, Hortelano S. 2011. Synthesis and anti-inflammatory activity of *ent*-kaurene derivatives. Eur J Org Chem 46: 1291–1305.

Llamozas S, Duno W, Meier R, Riina F, Stauffer G, Aymard O, Ortiz R. 2003. Libro rojo de la flora venezolana. PROVITA-Fundación Polar-Fundación Instituto Botánico de Venezuela Dr. Tobías Lasser, Caracas.

Meccia G, Quintero P, Rojas L, Usubillaga A, Carmona J. 2010. Análisis de los ácidos kaurénicos presentes en *Espeletiopsis angustifolia* Cuatrec. de Los Andes venezolanos. Avan Quím 5(1): 45–49.

Morillo G, Briceño B. 2007. Estudio sobre *Carramboa tachirensis* (Aristeg.) Cuatrec. (Asteraceae) y sus afines. Rev Fac Agron 24 (Supl 1): 475–481.

Morillo M, Rojas-Fermín LB, Aparicio R, Usubillaga A. 2017. *Ent*-Kaurene diterpenes and n-alkanes isolated from the leaves of *Coespeletia thyrsiformis* (A.C.SM.) Cuatrec. Biointerface Res Appl Chem 7(6): 2226–2229.

Nagashima F, Kondoh M, Kawase M, Simizu S, Osada H, Fujii M, Watanabe Y, Sato M, Asakawa Y. 2003. Apoptosis-inducing properties of *ent*-Kaurene-type diterpenoids from the Liverwort *Jungermannia truncata*. Planta Med 69(4): 377–379.

Obregón-Díaz YM, Rojas-Fermín LB, Usubillaga A, Pouységu L, Quideau S. 2015. Study of the chemical composition of the essential oil from the hybrid Asteraceae *Carramboa tachirensis* (Aristeg.) Cuatrec. Rev Fac Farm 57(1): 17–21.

Peña A, Alarcón L, Baptista J, Aparicio R, Villasmil T, Usubillaga A. 2012. A Phytochemical Analysis of *Espeletia nana* Cuatrec. a Midget Espeletiinae from Paramo Ortiz, Venezuela. Avan Quím 188 7(3):187–192.

Ríos N, Villalobos D, Rojas-Fermín L, Aparicio R, Usubillaga A, Mitaine-Offer AC, Lacaille-Dubois MA, Denis D, Peixoto Ph, Pouységu L, Quideau S. 2017. *In vivo* anti-inflammatory activity of grandiflorenic acid and kaurenic acid isolated from *Coespeletia moritziana* and Espeletia semiglobulata. Rev Fac Farm 59(1): 17–21.

Rojas LB, Gutiérrez R, Cordero de Rojas Y, Usubillaga A. 2008. Chemical composition of the essential oil from *Carramboa pittieri* (Cuatrec.) Cuatrec. (Asteraceae). Nat Prod Commun 3(10): 1739–1740.

Ruiz Y, Rodríguez J, Arvelo F, Usubillaga A, Monsalve M, Diez N, Galindo I. 2008. Cytotoxic and apoptosis-inducing effect of *ent*-15-oxo-kaur-16-en-19-oic acid, a derivative of grandiflorolic acid from *Espeletia schultzii*. Phytochemistry 69(2): 432–438.

Usubillaga A, Capra MC. 1988. Chemical constituyents of *Espeletia semiglobulata*. Fitoterapia 59(5):383.

Usubillaga A, Aparicio R, Romero M, Rojas, LB, Khouri N. 2001. Study of the essential oils from the leaves of four species of *Libanothamus* from the Venezuelan Andes. Flavour Fragrance J 16, 209–211.

Usubillaga A, Romero M, Aparicio R. 2003. Kaurenic acid in Espeletiinae. Acta Hortic 597:129–130.

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