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New Insights Into the Chemical Composition of *Baccharis palustris* Heering (Asteraceae) Essential Oil

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HIGHLIGHTS

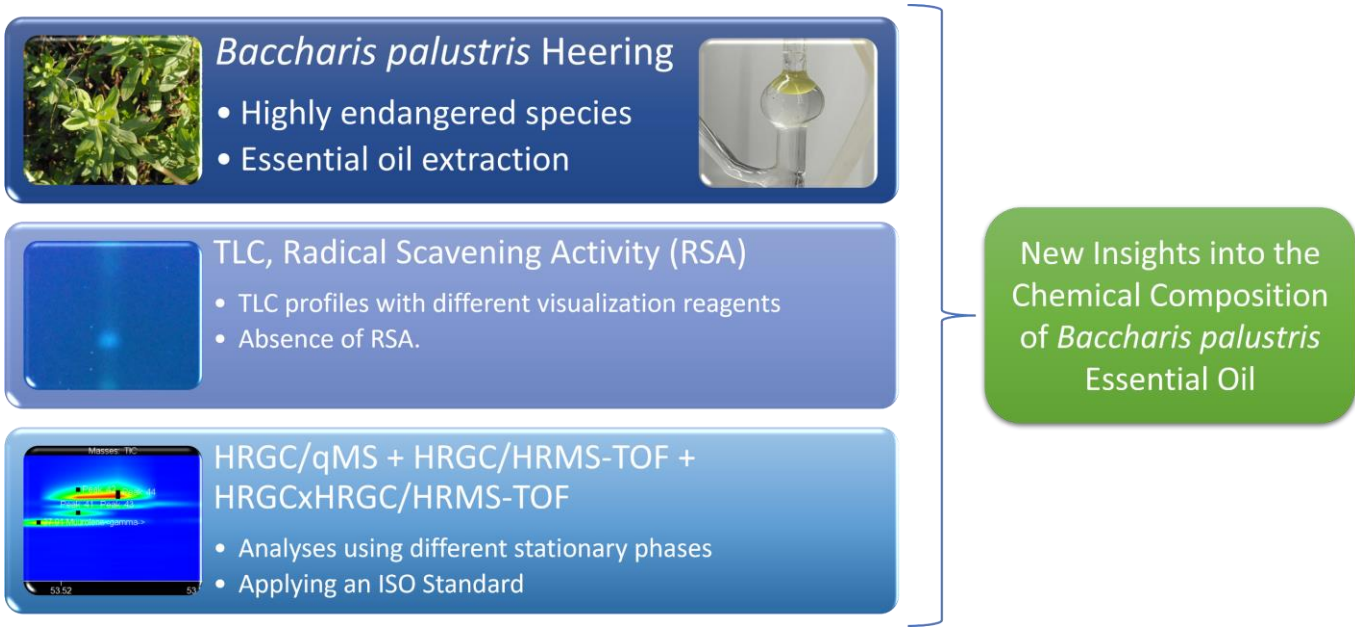
- TLC profiles of *B. palustris* essential oil with different visualization reagents.
- Original application of HRGCxHRGC/HRMS-TOF to a *Baccharis* spp. essential oil.
- Ten unreported terpenoids and lachnophyllum lactone identified in *B. palustris* oil.
- *B. palustris* oil did not exhibit DPPH radical scavenging activity.

Abstract: *B. palustris* Heering (Asteraceae), has been previously characterized as having an unusual essential oil composition with C_9-/C_{10}-polyacetylenes as main components, and mono- and sesquiterpenes/terpenoids at minor or trace levels. In this work, new insights into the chemical composition of this oil are presented: 1. TLC profiles using different visualization reagents for their characterization, 2. chemical analyses combining HRGC/qMS, HRGC/HRMS-TOF and HRGCxHRGC/HRMS-TOF, and 3. radical scavenging activity assay using the DPPH methodology were performed. The best TLC visualization conditions for the polyacetylenic components of the oil were obtaining using UV_λ= 365 nm and vanillin/H_3PO_4,

while the original application of NaDi (1-naphtol + *N*,*N*-dimethyl-*p*-phenylendiamine) demonstrated to be the best option to visualize the lachnophyllum acid methyl esters fraction. Gas chromatography/mass spectrometry protocols allowed the detection of 63 components in *B. palustris* oil: 39 of them were identified, 6 tentatively assigned without LRI information, and 18 could not be identified. Most of the identified components were mono- and sesquiterpenes and their derivatives. Ten of them are informed for the first time in *B. palustris* oil [α-pinene epoxide, rosefuran, *epi*-cubebol, cubebol, germacrene D-4-ol, junenol, *epi*-α-cadinol, *epi*-α-muurolol, germacra-4(15),5,10(14)-trien-1-β-ol and oplopanone]. C_9-/C_{10}-polyacetylenes (baccharisdyine/lachnophyllum acid derivatives) were confirmed as the main components of the oil, while other polyacetylenes were tentatively identified and their possible structures are discussed. The deconvolution analyses on HRGCxHRGC/HRMS-TOF allowed the identification of a lachnophyllum lactone isomer (undefined stereochemistry), co-eluting with the (*cis*)-lachnophyllum acid methyl ester peak. Finally, *B. palustris* oil was found to be an inactive DPPH radical scavenger.

Keywords: *Baccharis palustris*; essential oil; TLC, polyacetylenes; HRGCxHRGC/HRMS-TOF; DPPH.

GRAPHICAL ABSTRACT



INTRODUCTION

Baccharis L. is one of the largest genera belonging to Asteraceae represented to date by 442 accepted species plus at least 38 putative hybrids [1]. These species are considered as aromatic plants due to the presence of essential oils synthesized and stored in internal secretory ducts and superficial glandular trichomes of their aerial parts (leaves, branches, flowers, and fruits) [2]. However, currently only *B. dracunculifolia* DC. essential oil ("vassoura oil") is commercially available and marginally exploitable mainly for aromatherapy and flavor and fragrance industry [2,3]. In the last years, this oil has been confirmed as bioactive, with potential to control phytophatogenic fungi [4], human pathogenic bacteria [5,6], and eggs and larvae of bovine ticks (*Rhipicephalus microplus* Canestrini) [7]. Due to its commercial importance, *B. dracunculifolia* volatile composition has been extensively studied in the different geographical regions where it grows, and in relation to the environmental conditions, reproduction strategy (dioecism), phenological stages and seasonality [6,8,9].

Current research efforts on the study of *Baccharis* L. essential oils can be divided in two options, the first one intending to increase the knowledge and understanding regarding the potential applications of the "vassoura oil" (as the most prominent oil extracted from this genus). While in the other option, efforts are focused on to find *B. dracunculifolia* substituting species as a source of aromatic products with new potential applications. Recently, we described *B. uncinella* DC. as an alternative resource for obtaining products sensory related to the "vassoura oil" [10]. This election was based on the similar essential oil chemistry, dominated in both plant species by mono- and sesquiterpenes (hydrocarbons) and their derivatives, usually oxidized and/or rearranged (terpenoids) [9,10]. By contrast, a different situation is presented when a

completely different chemistry rules the essential oil composition, being thus expected different sensory properties and applications. This was the case in our recent report on a not previously chemically described species, *B. palustris* Heering, an endemic highly endangered shrub from Southern Brazil and Uruguay, whose oil presented an unusual composition with C_9/C_{10}-polyacetylenes as main components [11]. 1-Nonene-3,5-diyne (baccharisdiyne) represented up to 65.0% of the oil abundance, accompanied by two C_9-geometric isomers [1,7(*cis*)- and 1,7(*trans*)-nonadiene-3,5-diyne; or *dehydro*-baccharisdyines] which altogether summed around 20.2% [11]. In addition, two C_{10}-polyacetylene geometrical isomers: (*cis*)- and (*trans*)-lachnophyllum acid methyl esters were also identified, with a maximum abundance of 5.3% and 0.2%, respectively [11]. No other polyacetylenes were detected.

In the present work, we report new and useful information on the chemical composition of *B. palustris* essential oil, namely: 1. TLC profiles using different visualization reagents (highlighting the presence of polyacetylenic bands); 2. newly detected minor/trace-level components using a combination of HRGC/qMS, HRGC/HRMS-TOF and HRGCxHRGC/HRMS-TOF (HRGC/TOF and GCxGC/TOF for simplicity, respectively); and 3. DPPH radical scavenging activity (RSA) as a primary antioxidant screening method. To the best of our knowledge, this constitutes the first report on the use of GCxGC/TOF regarding to unravel the minor/trace-level components of *Baccharis* spp. L. essential oils.

MATERIALS AND METHODS

Plant Material and Essential Oil Extraction

Baccharis palustris aerial parts were collected at vegetative stage in a wetland environment at “Paso Carrasco” (Canelones, Uruguay) in July 2021. The plant sample was composed of several individuals, being representative of the entire population; seasonal aspects were investigated previously [11]. Taxonomical identification was conducted by H.A. González (National Museum of Natural History-NMNH, Montevideo), and a voucher sample was deposited at NMNH herbarium (MVM 23488 González). The sample was dry at 25°C in a shady, dry, ventilated room until reach constant weight. The essential oil was extracted from the plant material by hydrodistillation during 90 minutes with the aid of a modified Clevenger apparatus. After extraction, anhydrous sodium sulfate was added to the oil, filtered, and stored at refrigeration in amber glass vials; yield: 0.5% [11].

TLC analyses

B. palustris essential oil was diluted 1:99 in a mixture *n*-hexane-EtOH (1:1) (*n*-hexane 96%, Carlo Erba, Milan, Italy; EtOH ≥ 99.5%, Merck, Darmstadt, Germany) prior to apply 5.0 µL to the analytical plates (silica gel 60 F_{254}; Merck; 4 cm width x 10 cm height). To develop the analyses the optimized mobile phase was composed by CH_2Cl_2-*n*-hexane (2:1) (CH_2Cl_2 99.9%, Merck). TLC analyses were performed employing different visualization conditions (including the use of sprayer reagent solutions), as follows: **1.** $UV_{\lambda= 365\ nm}$, **2.** *p*-anisaldehyde/H_2SO_4, **3.** $CuSO_4$/H_3PO_4, **4.** NaDi, **5.** vanillin/H_3PO_4, **6.** MeOH/H_2SO_4, **7.** $UV_{\lambda= 254\ nm}$. Reagents **2.**, **5.** and **6.** were prepared according to Wagner & Bladt (1996) [12] with some slight modifications; while **3.** was based on EPFL (2018) [13], and **4.** is referenced from Ibanez and coauthors (2010) [14]. Briefly, the mixtures were performed as follows: **2.** 0.5 mL of *p*-anisaldehyde (for analysis; Fluka, Buchs, Switzerland) with 5.0 mL of H_2SO_4 (95-98%, Cicarelli, San Lorenzo, Argentina), 10.0 mL of acetic acid (glacial, 100%, Merck) and 85.0 mL of EtOH; **3.** 10.0 g of $CuSO_4$ (commercial quality, Paysandú Drugstore, Montevideo, Uruguay) with 100.0 mL of 10% aqueous solution of H_3PO_4 (85%, Merck); **4.** 1.0 g of 1-naphtol (for analysis, Merck) with 100.0 mL of EtOH (solution A) + 1.0 g *N*,*N*-dimethyl-*p*-phenylendiamine dihydrochloride (99.0%, Sigma-Aldrich, St. Louis, USA) with 100.0 mL of 1% aqueous solution of HCl (36.5-38.0%, Cicarelli) (solution B); **5.** 1.0 g of vanillin (Sigma-Aldrich) with 100.0 mL of 50% aqueous solution of H_3PO_4; and **6.** 90.0 mL of H_2SO_4 with 10.0 mL of MeOH (99.9%, Merck). For NaDi reagent (**4**), the solutions A and B were applied separately over the plates (in equal amounts), allowed them to react at room temperature, and immediately the displayed chromatograms were photographed. Almost five minutes after the application, the plate gained a deep blue color that made impossible to visualize the bands afterwards.

In parallel to the analysis of *B. palustris* oil sample, and for comparative purposes, two other *Baccharis* essential oils rich in mono and sesqui-terpenes/terpenoids were included: *B. trimera* (Less.) DC. [15] and *B. tridentata* Vahl. [16].

Preparative TLC was conducted on a 10 cm width x 10 cm height silica gel plate, where 80.0 µL of a solution 1:9 of *B. palustris* essential oil in a mixture of *n*-hexane-EtOH (1:1) was applied in an 8 cm wide band. After chromatogram developing, the plate edges were applied with the visualization reagent No. **5.** (vanillin/H_3PO_4), and subsequently a hydrocarbon (HPF) and one oxygenated fraction (LEF) were scrapped-

off from the plates (see Figure 1). The silica samples were extracted overnight with 5.0 mL of CH_2Cl_2. Then, both fractions were filtered, the solids were properly washed with CH_2Cl_2, and finally the fractions were evaporated to reduce the solvent volume to 2.0 mL for injection in a HRGC/qMS system.

HRGC/qMS, HRGC/TOF and GCxGC/TOF analyses

Baccharis palustris essential oil dilution (1:99) in *n*-hexane was submitted to injection in the GC systems. The analysis of this sample was performed accordingly to the ISO 7609:1985 standard reference [17]. Helium (99.9995% purity; Messer, Bogotá, Colombia; or Linde, Munich, Germany) at a 1.0 mL/min flow was employed as carrier gas in all the gas chromatography systems.

HRGC/qMS analyses were conducted on an Agilent 6890 Plus coupled to an MSD AT5793 Network instrument (Agilent Technologies, Santa Clara, USA), which was equipped sequentially with two capillary columns of different stationary phase polarities: ZB-5MS (60 m long x 0.25 mm ID x 0.25 µm df; Phenomenex, Torrance, USA; composition: 5%-diphenyl-95%-dimethylpolysiloxane) and DB-Wax (60 m long x 0.25 mm ID x 0.25 µm df; J&W Scientific, Folsom, USA; composition: 100% polyethylene glycol). In parallel, injections on a Shimadzu GCMS-QP2020 (Shimadzu Co., Kyoto, Japan) were performed on a Rxi-1MS (60 m long x 0.25 mm ID x 0.25 µm df; Restek, Bellefonte, USA; composition: 100%-dimethylpolysiloxane) to study the elution behavior of the main components of the oil and the composition of the TLC fractions obtained. The oven temperature conditions for ZB-5MS and DB-Wax analyses were the same as those reported previously by Minteguiaga and coauthors (2022) [11]; briefly: 40°C (4 min), 4°C/min, 180 °C (2 min), 10°C/min, 280 °C (10 min); and 40°C (4 min), 5°C/min, 180°C (0 min), 10°C/min, 220°C (10 min), 20°C/min, 240°C (10 min), respectively. For Rxi-1MS the oven was set as follows: 50°C (5 min), 5°C/min, 280°C (0 min). Injector temperatures: 250°C (ZB-5MS and DB-Wax) and 280°C (Rxi-1MS); kept constant. Interface temperatures: 230°C (DB-Wax), 300°C (ZB-5MS) and 280°C (Rxi-1MS); kept constant. Ionization chamber temperatures: 250°C, constant, in all the cases. Injection volumes: 1.0 or 2.0 µL; Split ratio: 1:30 (splitless in the case of the fractions obtained by TLC). Mass spectra were acquired to 70 eV electron ionization energy, working on full scan modality (45 to 450 m/z). Linear retention indices (LRI) were calculated for ZB-5MS and DB-Wax columns by performing the injection of a certified mixture of normal alkanes (C_6-C_{25}, AccuStandard, New Haven, USA) in the same chromatographic conditions as the original sample.

HRGC/TOF and GCxGC/TOF analyses were employed to confirm HRGC/qMS results and to find out possible new minor or trace components of the oil. The employed instrument was a Pegasus GC-HRT (Leco, St. Joseph, USA). When working as HRGC/TOF the capillary column was a DB-5MS (100 m long x 0.25 mm ID x 0.25 µm df; J&W Scientific; equivalent to the ZB-5MS) and all the other experimental chromatographic conditions were the same as those for HRGC/qMS analyses. When working as GCxGC/TOF, the 1st dimension (1D) was the above-mentioned DB-5MS column, while the 2nd dimension (2D) was equipped with a DB-17MS capillary column (2 m long x 0.25 mm ID x 0.25 µm df; J&W Scientific; composition: 50%-diphenyl-50%-dimethylpolysiloxane). A cryogenic dual jet/loop modulator was employed to connect 1D to 2D; modulation time: 5 s.

The identification or tentative assignation of the essential oil components including the minor or trace components was achieved by comparison with different commercial mass spectral libraries [18-20], with LRI databases [20-22] and with our previous publication data [11].

DPPH Radical Scavenging Activity (RSA)

A 0.30 mg/mL DPPH radical (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich) mother solution was prepared in EtOH 96.0% (Dorwil, Buenos Aires, Argentina), covering the flask with an aluminum foil to avoid its sunlight degradation [23]. For the analytic procedure, the protocol described by Koleva and coauthors (2002) [23] was followed with some modifications based on the experimental conditions. An aliquot of 5.0 µL of *B. palustris* essential oil was diluted with 995 µL of EtOH, and then mixed with 1000 µL of the DPPH mother solution in a quartz cuvette (final concentration: 2.5 µL/mL). Immediately, the absorbance (A) was recorded at 517nm using a UV-Vis spectrophotometer (Metrolab VD-40, Metrolab, Buenos Aires, Argentina) considering as t_0 the first measurement, and then registering the A every one minute up to a total time of 30 minutes. As positive controls of RSA pure standards of eugenol and carvacrol were employed, while the negatives ones were estragole, *p*-cymene and α-pinene (all of them provided by Sigma-Aldrich); preparation of the solutions was performed as above described. For comparison purposes, dilutions of pure *B. trimera* and *B. tridentata* essential oils were also used and prepared as above mentioned.

For the DPPH radical scavenging activity calculation, the inhibition percentage (I) parameter was employed (sometimes mentioned in the literature as the quenching percentage), calculated as follows [23]:

$$Inhibition\ \%\ (I)\ =\ 100\ [(A_B - A_{(S\ +\ DPPH,\ t)})/A_B]$$

Where A_B was the DPPH working solution absorbance (1000 µL of the DPPH mother solution + 1000 µL of EtOH), and $A_{(S + DPPH, t)}$ was the respective value of the mixed solution for the measurement (1000 µL of the mother DPPH solution + 5.0 µL of essential oil/standard + 995 µL of EtOH) at 30 minutes. All the determinations were conducted in triplicate in the same day, monitoring constantly that A_B value was stable during the procedure.

RESULTS

TLC analyses

TLC analysis was developed as a fast analytical tool to visualize the chemical composition of *B. palustris* essential oil, as well as to easily locate the corresponding polyacetylenic bands in the plates. Figure 1 shows the recorded TLC chromatograms.

Preparative TLC followed by HRGC/qMS analyses allowed to confirm that the two bands highlighted in the Figure 1 corresponded to the hydrocarbon polyacetylenic fraction (HPF) or the lachnophyllum methyl esters fraction (LEF) (Figure S1 and S2).

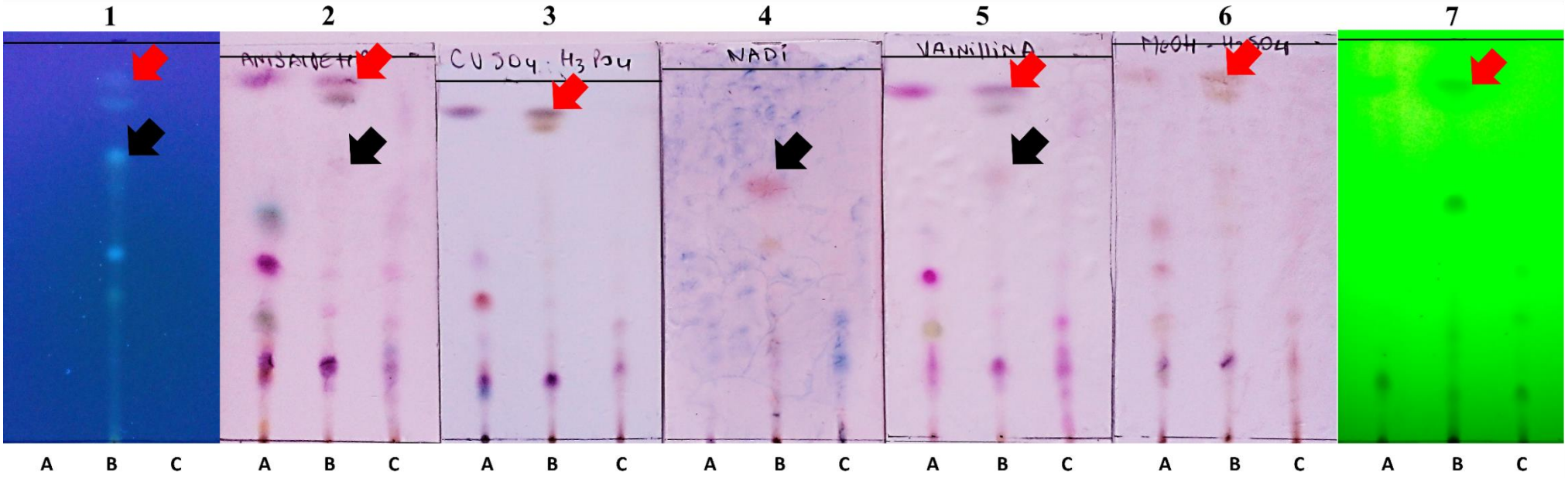


Figure 1. TLC analyses results for *B. palustris* essential oil 1:99 dilution (B) with different spray visualization reagents or UV light (**1.** $UV_{\lambda= 365\ nm}$; **2.** *p*-anisaldehyde/H_2SO_4, **3.** $CuSO_4$/ H_3PO_4; **4.**, NaDi; **5.** vanillin/H_3PO_4; **6.**, MeOH/H_2SO_4; **7.** $UV_{\lambda= 254\ nm}$). The band corresponding to the hydrocarbon polyacetylenic fraction (HPF; Rf: 0.93; including two sub-bands in some cases) is indicated using a red arrow, while the black arrow represents the lachnophyllum acid methyl esters fraction (LEF; Rf: 0.70), according to preparative TLC followed of HRGC/qMS analyses. For comparative purposes, there were employed essential oil samples of *B. trimera* (A) and *B. tridentata* (C) [15,16] at the same dilution level.

HRGC/qMS and HRGC/TOF analyses

Table 1 summarizes the components found for *B. palustris* essential oil. Through application of the ISO standard (1985) [17] criteria to analyze this sample by HRGC/qMS, HRGC/TOF and GCxGC/TOF, 39 components were identified (I_1-I_{39}) by LRI confirmation in one or two different stationary phases (ZB-5MS and DB-Wax), 6 were tentatively identified (T_1-T_6; including two putative geometrical isomers) without LRI confirmation due to the literature paucity, while 18 components (U_1-U_{18}) could not be identified but some information regarding molecular formulas and mass spectra is provided. Thus, 63 compounds were detected in this study, compared to the 51 components previously informed [11]. Confirming former results, the *B. palustris* essential oil's most abundant components were C_9-polyacetylenes, namely: baccharisdyine (56.3%-63.1%), 7-(*cis*)-*dehydro*-baccharisdyine (13.4-15.8%), and 7-(*trans*)-*dehydro*-baccharisdyine (1.6-2.1%), accompanied by the C_{10}-polyacetylene *cis*-lachnophyllum acid methyl ester (9.5-10.3%). Figure S1 illustrate a representative HRGC/qMS chromatogram profile of the sample.

As shown in Table 1, several of the components (especially those at minor or trace-level) co-eluted and, therefore, would not be identified through gas chromatography protocols using only one stationary phase (one chromatographic dimension, 1D). Thus, the present results highlight the importance of employing orthogonal phases (as ZB-5MS and DB-Wax) in complementary HRGC/qMS or HRGC/TOF analyses. As well as the application of a second chromatographic dimension (2D; through GCxGC/TOF) in order to increase the number of components identified in complex essential oils, as the present one.

Table 1. Components of *B. palustris* oil: identified (I_1-I_{39}), tentatively identified (T_1-T_6) and unidentified (U_1-U_{18}).

		LRI - ZB-5MS[1]		LRI - DB-Wax[1]		% Abund.		
Tag	**Components** (ordered by elution in ZB-5MS or in DB-Wax)	**Exp.**	**Lit.**	**Exp.**	**Lit.**	**DB-5MS**	**DB-Wax**	**Pr. Inf.***
I_1	α-Pinene	931	932	nd	1036	tr	nd	Yes
U_1	C_9H_{12}: 120(52), 105(26), 91(51), 78(100), 65(46)	946	-	nd	-	tr	-	No
T_1	1-Nonen-3-yne	953	-	1161	-	tr	tr	No
U_2	C_8H_8: 104(100), 78(54), 77(52), 51(17)	971	-	d	-	tr	-	No
I_2	β-Pinene	974	974	1090	1092$^{\Delta}$	0.1	tr	Yes
I_3	Myrcene	990	988	1154	1156	0.1	tr	Yes
$T_2 + T_3$	**3-Ethylidene-2-methyl-1-hexen-4-yne**^{I}	1005^{x}	-	1289	-	1.1^{I}	0.7^{I}	No
I_4	Limonene	1026	1024	1184	1187$^{\Delta}$	0.1	tr	Yes
U_3	C_9H_{12}: 120(34), 105(14), 91(100), 78(67), 65(35)	nd	-	1193	-	-	tr	No
I_5	(*cis*)-β-Ocimene	1036	1032	1234	1256$^{\Delta}$	0.1	0.1	Yes
I_6	(*trans*)-β-Ocimene	1047	1044	1249	1250	4.2	3.0	Yes
U_4	$C_{10}H_{16}O$: 137(3), 109(9), 95(21), 83(77), 67(100), 41(57)	nd	-	1384	-	-	0.1	No
I_7	**Baccharisdyine**	1085	1089*	1443	1468*	56.3	63.1$^{\#}$	Yes
I_8	**7-(*cis*)-*Dehydro*-baccharisdyine**	1093	1097*	1504	1522*	15.8	13.4	Yes
I_9	Rosefuran	1097	1093$^{\Delta}$	nd	1413$^{\Delta}$	0.1	nd	No
I_{10}	α-Pinene oxide	1098	1099	1349	1353$^{\Delta}$	0.2	0.1	No
I_{11}	Linalool	1099	1095	1536	1550$^{\Delta}$	0.1	0.1$^{\$}$	Yes
U_5	**C_8H_8O**; MW: 120.0570; Δ: 0.02 ppm	1108	-	1608	-	0.7	0.6	No
T_4	3-Hydroxyphenylacetylene	1118	-	-	-	0.1	nd	No
I_{12}	**7-(*trans*)-*Dehydro*-baccharisdyine**	1122	1121*	1581	1571*	2.1	1.6	Yes
I_{13}	(*trans*)-β-Ocimene epoxide [(*trans*)-myroxide]	1143	1140	1464	1479$^{\Delta}$	0.1	0.1	Yes
U_6	132(5), 115(15), 88(100), 62(39), 41(11)	1206	-	nd	-	0.1	-	No
U_7	134(6), 105(100), 91(11), 77(17), 51(19)	1237	-	nd	-	0.1	-	No
T_5	3-Phenyl-2-propyn-1-ol	1249	-	-	-	0.1	nd	No
$U_8 + U_9$	**$C_9H_{10}O + C_9H_{10}$.** $C_9H_{10}O$; MW: 134.0726; Δ: 0.40 ppm	1267	-	nd	-	0.4	-	No
U_{10}	$C_{10}H_{18}$: 138(19), 123(75), 95(100), 82(55), 67(64), 41(54)	nd	-	1519	-	-	0.1	No

Cont. Table1

Tag	**Components** (ordered by elution in ZB-5MS or in DB-Wax)	**LRI - ZB-5MS[1]**		**LRI - DB-Wax[1]**		**% Abund.**		
		Exp.	**Lit.**	**Exp.**	**Lit.**	**DB-5MS**	**DB-Wax**	**Pr. Inf.***
I_{14}	α-Copaene	1375	1374	1474	1519	0.3	0.2	Yes
I_{15} + I_{16}	β-Cubebene + β-elemene	1391	1387/1389	1523/ 1571	1541/ 1591	0.2	0.1/0.1[$]	Yes
I_{17}	(*trans*)-β-Caryophyllene	1419	1417	1577	1589[Δ]	0.3	0.3	Yes
U_{11}	152(2), 134(6), 121(100), 103(12), 91(44), 77(44)	1465	-	Nd	-	0.2	-	No
I_{18}	Germacrene D	1484	1484	1681	1683[Δ]	0.6	0.5	Yes
U_{12}	**$C_{11}H_{14}O_2$**; MW: 178.0986; Δ: 1.51 ppm**.** 178 (51), 163 (7), 150 (38), 135 (38), 117 (38), 107 (82), 91 (100), 77 (79)	1485	-	2121[@]	-	0.2	0.2[@]	No
I_{19} + I_{20}	Bicyclogermacrene + *epi*-cubebol	1497	1500/1493	1705/1865	1699/2088[Δ]	0.7	0.1/0.3	Yes/No
I_{21}	(*trans*)-Lachnophyllum acid methyl ester	1508	1534[*]	2083	na	0.2	0.3	Yes
I_{22} + I_{23}	**(*cis*)-Lachnophyllum acid methyl ester + lachnophyllum lactone[§]**	1516	1519[*]/1492[Δ]	2208	na/na	9.5/ tr	10.3/ tr	Yes
I_{24} + I_{25}	δ-Cadineno + cubebol	1523	1522/1514	1734/1912	1785/ 1930	0.6	0.3/0.2	Yes/No
I_{26}	*trans*-Nerolidol	1563	1561	2010	2000	0.4	0.3	Yes
I_{27}	Palustrol	1568	1567	1903	1938[Δ]	0.2	0.1	Yes
I_{28} + I_{29}	Spathulenol + germacrene D-4-ol	1579	1577/1574	2094/2017	2110/2035[Δ]	2.0	1.5/0.2	Yes/No
I_{30} + U_{12}	Caryophyllene oxide + sesquiterpenoid $C_{15}H_{24}O$: 220(4), 202(21), 187(49), 159(66), 119(45), 96(68), 91(65), 43(100)	1584	1582/na	1950/2117	1966/na	0.6	0.2/0.2	Yes/No
I_{31}	Viridiflorol	1594	1592	2056	2081[Δ]	0.2	0.1	Yes
I_{32}	Junenol	1619	1618	nd	2052[Δ]	0.1	nd	No
I_{33}	*iso*-Spathulenol	1637	1638[Δ]	nd	2225[Δ]	0.2	nd	Yes
I_{34}	*epi*-α-Cadinol (ζ-cadinol)	1644	1638	nd	2162[Δ]	0.1	nd	No
I_{35}	*epi*-α-Muurolol (ζ-muurolol)	1645	1640	nd	2187[Δ]	0.2	nd	No
I_{36}	α-Muurolol (δ-Cadinol)	1650	1644	2174	2150	0.2	0.1	Yes
U_{13}	Sesquiterpenoid $C_{15}H_{26}O$: 222(3), 207(17), 204(24), 161(90), 133(45), 95(100), 43(90)	nd	-	2154	-	-	0.1	No
I_{37}	α-Cadinol	1653	1652	2200	2224	0.3	0.3	Yes
U_{14}	Sesquiterpenoid $C_{15}H_{24}O$: 220(17), 202(28), 187(47), 159(49), 107(100), 93(85), 69(31)	1679	-	2121[@]	-	0.1	0.2[@]	No
I_{38}	Germacra-4(15),5,10(14)-trien-1-β-ol	1690	1685	2331	na	0.2	0.2	No
I_{39}	Oplopanone	nd[&]	1739	2488	2545	nd	0.1	No
U_{15}	236(46), 221(3), 207(100), 191(66), 178(51), 165(49), 115(18), 91(11)	1980	-	nd	-	0.1	-	No

Cont. Table 1

T_6	Methyl-5,8,11-heptadecatriynoate	2001	-	2424	-	0.2	0.1	No
U_{16}	254(9), 239(14), 211(29), 155(40), 128(58), 93(100)	nd	-	2436	-	-	0.3	No
U_{17}	236(100), 221(4), 207(58), 191(38), 178(41), 165(36), 152(19)	2119	-	nd	-	0.1	-	No
U_{18}	134(5), 121(100), 103(15), 91(42), 77(53)	nd	-	2840•	-	-	0.4	No

[1]Exp. LRIs and Lit. LRIs: experimental retention indices obtained in the corresponding column or from the literature respectively {unless other reference be cited, Adams (2017) [20] for ZB-5MS and Davies (1990) [21] for DB-Wax}. References: (bold) components analyzed by HRGC/qMS and HRGC/TOF; (na) not available; (nd) not detected; (tr): traces (abundance <0.05%); (*) previously informed in Minteguiaga and coauthors (2022) [11]; (^{x}) LRI_{DB-5} = 1070 from Razavi (2012) [24]; (!) reported as "unidentified" in Minteguiaga and coauthors (2022) [11], two putative geometrical isomers (see GCxGC/TOF analyses); (#) co-elution with a compound C_9H_8; (@) co-elution in DB-Wax; ($) in the polar column- linalool and β-cubebene co-eluted; (&) LRI_{HP-5MS} = 1742 according to Minteguiaga and coauthors (2018) [25]; (Δ) LRI values from NIST Webbook (as informed by the authors: normal alkane retention index) [22]; (§) lachnophyllum lactone having unknown stereochemistry (see GCxGC/TOF analyses); (•) value outside the normal alkane solution (C_8-C_{25}) retention range. For the unidentified components, the intensities of the ion fragments of the corresponding mass spectra (EI, 70eV) in low- mass resolution are presented as: m/z, I%; when possible, high- mass resolution information is provided.

GCxGC/TOF analyses

To the best of our knowledge, this is the first report on the *Baccharis* L. genus regarding the employment of GCxGC/TOF. Figures 2 and 3 present contour plots corresponding to co-elutions in the first dimension of gas chromatography analyses (1D) which were resolved in the second dimension (2D) employing GCxGC/TOF. Thus, some of the corresponding peaks were not detected by HRGC/qMS nor HRGC/TOF.

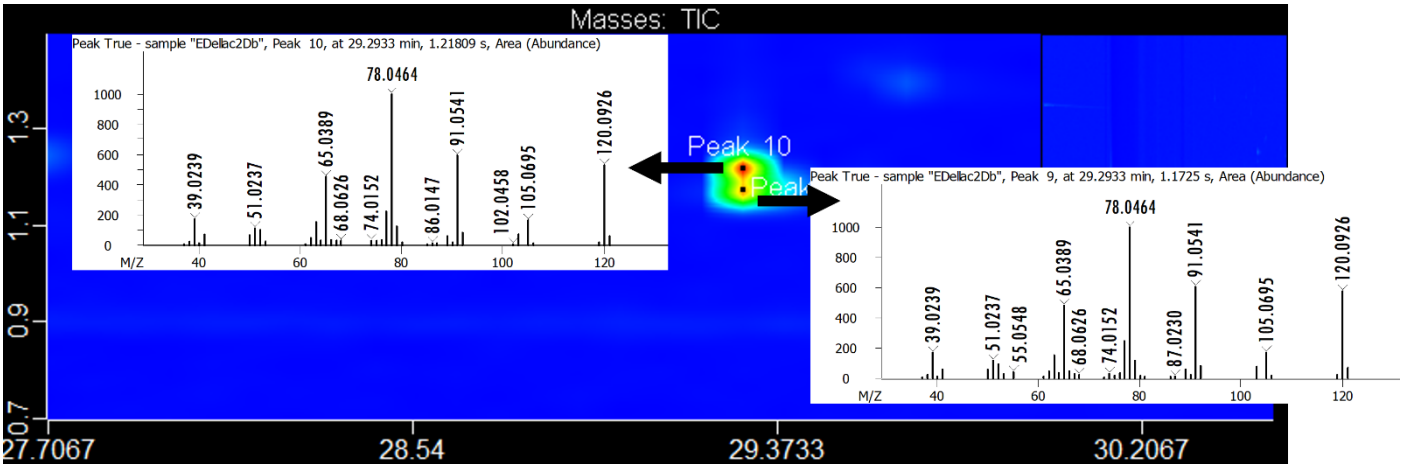


Figure 2. GCxGC/TOF contour plot and mass spectra corresponding to the co-elution of peaks #9 and #10 of *B. palustris* essential oil, putatively assigned to the geometric isomers of 3-ethylidene-2-methyl-1-hexen-4-yne (EMHs).

Figure 2 shows the peaks of two putatively assigned geometric isomers of 3-ethylidene-2-methyl-1-hexen-4-yne (EMHs), while in Figure 3 co-elution of *cis*-lachnophyllum acid methyl ester and lachnophyllum lactone (LL) is observed. No information regarding the stereochemistry of the double bonds was obtained for EMHs and LL.

Other co-elutions, with unidentified components were detected. The corresponding GCxGC/TOF contour plots are presented as supplementary materials (Figures S3 to S7).

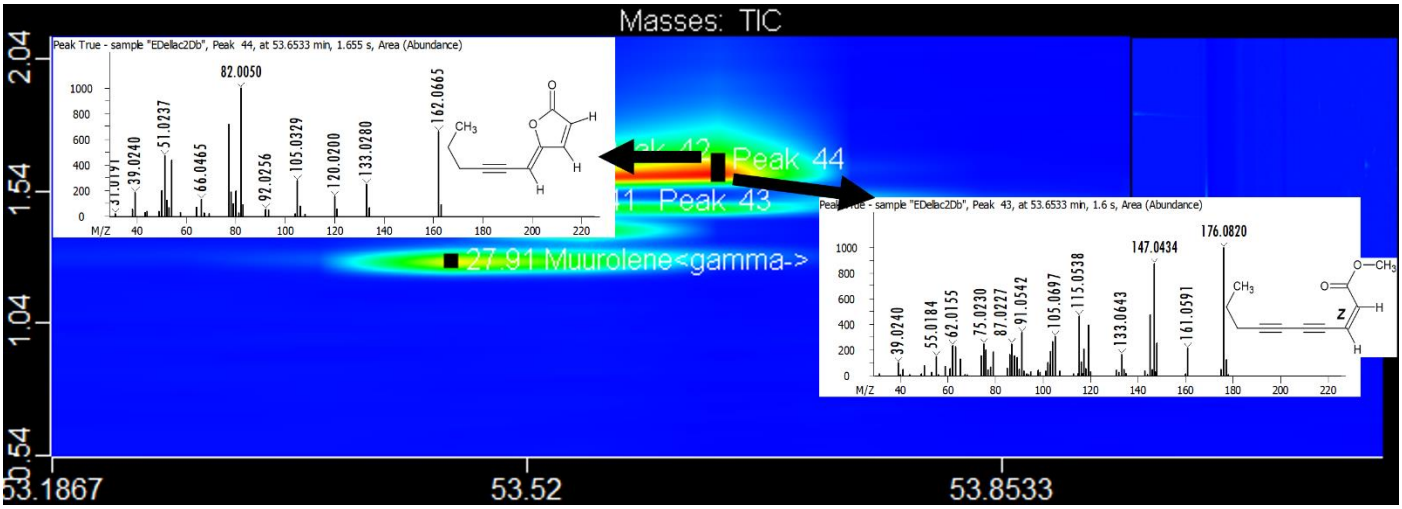


Figure 3. GCxGC/TOF contour plot and mass spectra corresponding to the co-elution of peak #43 [(*cis*)-lachnophyllum acid methyl ester] and peak #44 (lachnophyllum lactone; unknown stereochemistry) from *B. palustris* essential oil.

DPPH Radical Scavenging Activity (RSA)

Table 2 shows the RSA of *B. palustris* and other related *Baccharis* L. essential oils employed as references for this work. As indicated, no activity (I: 0%) was evidenced for the studied sample, while the other essential oils employed as reference were marginally active (I: 1.6% for *B. tridentata* and 3.0% for *B. trimera* oils). This assay was confirmed as valid since the results for the standards selected as positive (eugenol and carvacrol, I: 55.0% and 86.5%, respectively) and negative controls (α-pinene, *p*-cymene and estragole, I: 0% for all of them) were according to the expected.

Table 2. DPPH radical scavenging activity for *B. palustris* essential oil, other related *Baccharis* spp. oils and the standard controls: positives (eugenol and carvacrol), and negatives (α-pinene, *p*-cymene and estragole).

Samples	Inhibition % (I)[1]
B. trimera	3.0
B. palustris	**0**
B. tridentata	1.6
estragole	0
α-pinene	0
p-cymene	0
eugenol	55.0
carvacrol	86.5

[1] After 30 min of evaluation; concentration: 2.5 µL of oil or standard/mL (in EtOH).

DISCUSSION

TLC analyses

In a previous study, we established the unusual composition of *B. palustris* essential oil, with a polyacetylene abundance ranging from 75.0% to 88.8% [11]. In that work, TLC analysis of such a sample presented some visualization difficulties as the band corresponding to the lachnophyllum acid methyl ester fraction (LEF: 4.5% of abundance: composed of the *cis*- and *trans*-isomers) was scarcely perceived with the *p*-anisaldehyde/H_2SO_4 visualization reagent. To resolve this limitation and with the aim to be employed as a standard, a previous isolation and structural elucidation of the (*cis*)-lachnophyllum acid methyl ester from *Erigeron bonariensis* L. [synonym: *Conyza bonariensis* (L.) Cronquist] was conducted [11]. In the current work, we explored more in deep the conditions to better visualize the LEF band in TLC, finding the best visualization trade-off for all the components of the sample (specially the polyacetylenes). According to the Figure 1, UV_λ= 365 nm and vanillin/H_3PO_4 constituted the best conditions to visualize both, the hydrocarbon polyacetylenic fraction (HPF; Rf: 0.93) and the LEF fraction (Rf: 0.70), being that with the employment of UV the two bands were exhibited as more conspicuous. As expected, *B. trimera* and *B. tridentata* essential oils did not present any absorption in UV_λ= 365 nm due to the absence of polyacetylenes in their compositions [15,16]. In agreement with our previous work, *p*-anisaldehyde/H_2SO_4 cannot allow a good visualization of the LEF band (Figure 1). Interestingly, NaDi reagent (1-naphtol + *N*,*N*-dimethyl-*p*-phenylendiamine) demonstrated to be the best option to visualize the latter (black arrows in Figure 1), but no reaction with the HPF band was observed. All the other reagents employed were unsuccessful to visualize the LEF band, while allowing the detection of the HPF one (Figure 1). These results are relevant since polyacetylenes are generally recognized as a chemical group hardly to be visualized in TLC, being considered in the literature the UV and acidic solutions of vainillin and *p*-dimethylaminobenzaldehyde as the more suitable spray reagents useful to visualize these compounds (including the LEF isomers) [26].

NaDi stain mixture was originally introduced for the histochemical analyses in animal tissues, and afterwards, it was proposed as a dye for the cytochrome oxidase activity localization [27]. Eventually, NaDi applicability was expanded to localize lipids and terpenes (preferably, mono- and sesquiterpenes composing essential oils) in vegetal samples [14]. When NaDi is applied to the tissues it oxidizes yielding a blue coloration due to the formation of indophenol [27]. To our knowledge this is the first time that a NaDi mixture is employed as spray reagent for TLC visualization, being informed here as a promising methodology to visualize the LEF band with a good intensity at the concentration worked (Figure 1).

HRGC/qMS, HRGC/TOF and GCxGC/TOF analyses

As it was above described, the main components of *B. palustris* essential oil were polyacetylenes (more than 88.0% of abundance, Table 1). However, most of the identified components (in terms of number of them) belong to the terpene/terpenoid families (mono- and sesqui-), but usually they occur as minor or trace-level components (<1.0% of abundance); being the only exceptions (*trans*)-β-ocimene (3.0-4.2%) and spathulenol (1.5-2.0%). Other compounds highlighted by their abundance were bicyclogermacrene (0.1-0.7%), δ-cadinene (0.3-0.6%) and caryophyllene oxide (0.2-0.6%). This composition is extremely unusual for an essential oil obtained by hydrodistillation or steam distillation from aerial parts of *Baccharis* spp. [2]. In fact,

these oils are frequently composed by terpenes/terpenoids as the main components (concentrating in general sesqui- over mono-), and phenylpropanoids, lineal and branched aliphatics and some simple aromatics as minor or trace components [2]. However, there are some exceptions to these general trends, i.e.: *B. reticularioides* Deble & A.S.Oliveira (with α-pinene as the main component, reaching *ca.* 25% of abundance) [28], *B. darwinii* Hook. & Arn. and *B. heterophylla* Kunth exhibiting approximately 47% and 36% of limonene abundance, respectively [29,30], and notably *B. trimera* (Less). DC. which oil can concentrate up to 70% of the irregular monoterpene carquejyl acetate at blooming stage [15]. In other species, such as *B. pauciflosculosa* DC., *B. punctulata* DC. and *B. sphenophylla* Herring & Dusén almost an equal ratio between mono- and sesqui- terpenes/terpenoids was informed [28]. The occurrence of polyacetylenes in *Baccharis* L. is interesting from an evolutionary point of view, as well as significant to ecological level. No other *Baccharis* spp. is comparable to *B. palustris* in terms of the production of C_9-/C_{10}-polyacetylenes. Apart of this species, the presence of polyacetylenes in its essential oil was described only for *B. trinervis* (Lam.) Pers. (the two isomers of lachnophyllum acid methyl ester) [31].

Ten terpenoids not previously reported for *B. palustris* were detected in this study (abundance ≤0.3%; Figure S8 and Table 1) after HRGC/qMS and HRGC/TOF analyses, namely: α-pinene oxide, rosefuran, junenol, germacra-4(15),5,10(14)-trien-1-β-ol, germacrene D-4-ol, cubebol, *epi*-cubebol, *epi*-α-cadinol, *epi*-α-muurolol and oplopanone. All of them have been previously found in the volatile fractions/extracts of other *Baccharis* spp. Namely, α-pinene oxide has been detected in *B. latifolia* Pers. [32], *B. darwinii* [29] and *B. uncinella* [10]; and rosefuran in *B. trimera* [15]. Oplopanone was informed in the extracts of aerial parts of *B. uncinella* [10], *B. sphenophylla* Dusén ex Malme [33], and *B. gnaphalioides* DC. [25]. Junenol was identified previously in *B. articulata* (Lam.) Pers. [34], *B. dracunculifolia* [8,35] and *B. trinervis* Pers. [36]. Cubebol was determined in *B. oreophila* Malme [37] and *B. trinervis* [36], while *epi*-cubebol was identified in *B. gnaphalioides* [25]. Germacra-4(15),5,10(14)-trien-1-β-ol was reported in the commercial "vassoura oil" [35], while germacrene D-4-ol was detected in *B. articulata* [34]. Finally, *epi*-α-cadinol (synonym: ζ-cadinol) and *epi*-α-muurolol (synonym: ζ-muurolol) in general appear together in the essential oils and have been identified previously in a wide range of species, among them, *B. articulata* [34], *B. salicifolia* and *B. dracunculifolia* [32]. In addition to the named components, during GCxGC/TOF deconvolution experiments it was possible to detect a compound tentatively identified as *epi*-α-cadinol acetate (or ζ-cadinol acetate), but this compound was not included in Table 1 because no LRI values from the literature were found. Its putative mass spectrum is presented as supplementary material (Figure S7).

In this work, polyacetylenes were confirmed as the main components of *B. palustris* essential oil by HRGC/TOF and HRGC/qMS (Table 1, Figure S9): baccharisdyine (**11**), 7-*dehydro*-baccharisdyine geometric isomers (**12** and **13**), and (*cis*)-lachnophyllum acid methyl ester (**14**) co-eluting with a lachnophyllum lactone isomer (**15**) (Figure 3). Six other minor or trace-level polyacetylenes were tentatively identified after a software comparison with commercial mass spectral libraries in low- or high-mass resolution experiments: **16** to **21** (Table 1 and Figure S9). 1-Nonen-3-yne (**16**), 3-ethylidene-2-methyl-1-hexen-4-yne (**17** and **18**; EMH), 3-hydroxyphenylacetylene (**19**), 3-phenyl-2-propyn-1-ol (**20**), and methyl-5,8,11-heptadecatriynoate (**21**) were tentatively identified by HRGC/qMS and/or HRGC/TOF analyses. From them, the structure of 1-nonen-3-yne is perfectly aligned with the corresponding of baccharisdyine, being the difference only a simple bond instead of a triple one at position 5 of the carbon chain (Figure S9). Whilst the putative identification of the two EMH geometric isomers was obtained after HRGC/TOF and GCxGC/TOF analyses (C_9H_{12}, m/z: 118.0775, Δ: 0.60 ppm) (Figures 2 and S9). The presence of these two possible geometric isomers of EMH was evidenced by GCxGC/TOF, upon that the second dimension demonstrated two peaks with essentially the same mass spectrum (Figure 2). EHM (**17**,**18**) was previously reported as a main component of other essential oils, *i.e.*, *Prangos acaulis* Bornm [38] and *P. ferulaceae* L. (Apiaceae) [24]. To the best of our knowledge, 1-nonen-3-yne (**16**), 3-hydroxyphenylacetylene (**19**) and 3-phenyl-2-propyn-1-ol (**20**) have not been previously reported in essential oils to date. The putative presence of methyl-5,8,11-heptadecatriynoate (**21**) is relevant since it is a derived ester from a polyacetylenic fatty acid. This compound has been recently informed as a component of the supercritical fluid extract of two Chinese Apiaceae traditional herbs [39], but not reports have been found about its occurrence in essential oils. The biosynthetic pathway of polyacetylenes proceeds starting from linoleic acid and, through the action of an acetylenase, the crepenynic acid is obtained [40]. From this latter compound, a pool of polyacetylenes is constructed, including other polyacetylene fatty acids [40]. Thus, the putative occurrence of **21** in *B. palustris* could mean, from a metabolomic point of view, a great degree of activation of the crepenynic pathway. The real position of the triple bonds in the carbonated chain could not be confirmed with the present research approach, and new experiments are needed to confirm these positions.

Previously, we found out that HRGC/qMS is not a tool enough for a complete chemical constituents' description of *B. palustris* oil. In fact, the software comparison with mass spectral libraries provided 97% of matching with 1-phenyl-1-propyne (**22,** Figure S9) for the corresponding peak of 7-(*cis*)-*dehydro*-baccharisdyine (**12,** Figure S9), but co-injection and FT/IR experiments discarded this possibility [11]. Thus, following the same rationale, and considering that no standards were available for compounds **16** to **21** (Figure S9), here we hypothesize structures more related to baccharisdyine as alternatives for these components, until new full structural elucidation experiments can be executed. For example, 3-phenyl-2-propyn-1-ol (**20**) has the same skeleton as 1-phenyl-1-propyne (**22**), and then, an alternative structure might be like **23**, despite (for sure) the position of the hydroxyl group can vary at both ends of the molecule. Regarding EHM, the tentative presence of both isomers in *B. palustris* essential oil is questionable as the only LRI value available from the literature do not fit very well (1005 *vs.* 1070) [24]. Thus, other C_9H_{12} structures more related to that of baccharisdyine (**11**) as those presented as **24** and **25** (Figure S9) cannot be ruled out. Other structural motifs with interchangeable positions of the double and triple bonds are also alternatives. Previously we hypothesized that C_9-polyacetylenes **11** to **13** could be associated to a biosynthetic scheme starting from C_{10}-polyactetylenes as precursors, trough the activity of hydrolases, descarboxylases and desaturases (for the conversion of **11** to **12** and **13**) [11]. The presence of **16**, and **23-25** could be related to similar biogenetic origins.

A surprising result was obtained after GCxGC/TOF analyses of the (*cis*)-lachnophyllum acid methyl ester peak, which was able to be resolved into two peaks in the second chromatographic dimension, the second one with the spectrum of a lachnophyllum lactone (**15,** Figure S9) of undefined stereochemistry (Figure 3). This compound rendered the characteristic even ion at m/z 82.0050 as base peak, which is originated from the lactone ring cleavage [41]. Lachnophyllum lactone has been reported in the literature as a bioactive component with allelopathic [42], fungitoxic [43] and repellent properties [44]. The presence of this compound in *B. palustris* essential oil even at trace level, might contribute to its potential bioactivity, which need to be evaluated further. Polyacetylenes are interesting compounds from pharmacological and phytotherapeutic points of view, as they exhibit promising cytotoxic and antitumoral activities, *i.e.*, C_{17}-polyacetylenes such as falcarinol (panaxynol), panaxydol and panaxytriol from *Panax ginseng* C.A. Meyer (Araliaceae) [45].

Several components of *B. palustris* oil could not be identified in this work (Table 1), but the information obtained gives some clues about their chemical nature. For instance, the component with $LRI_{ZB\text{-}5MS}$ = 1108 (U_5; abundance: 0.7%) corresponds to a C_8H_8O formula possibly being an aromatic derivative, as the most probable compound in the databases for this peak was 2-phenylacetaldehyde (but no LRI confirmation was obtained). Furthermore, the possibility of C_8-polyacetylenes occurrence cannot be ruled out since 3-hydroxyphenylacetylene (**19**) was tentatively assigned. Moreover, several of the structural formulae shown in Table 1 presents a high degree of unsaturation, which suggests the presence of more polyacetylenes. So far, *B. palustris* represents a promising species to continue under study as a model of low molecular weight polyacetylene biosynthesis.

DPPH Radical Scavenging Activity (RSA)

Polyacetylenes in general are associated with a strong antioxidant activity, including high level of RSA on DPPH tests. For example, Dumlu and coauthors (2008) [46] reported on the isolation of two C_{14}-polyacetylenes (lobetyol and lobetyolin) from *Campanula alliariifolia* Willd. (Campanulaceae) which exhibited a good level of activity (I: 82%-98%). In line, a high abundance of falcarinol in *Eryngium pseudothoriifolium* Contandr. & Quézel (Apiaceae) essential oil (84.0%) has been correlated with higher RSA when compared to *E. thoriifolium* Boiss. oil, which in turns do not present this compound [47]. Thus, *B. palustris* essential oil rich in C_9-polyacetylenes was submitted to the DPPH test to evaluate if these compounds also presented a good RSA. According to the results presented in the Table 2, no activity was evidenced, and marginal activity was found for the other *Baccharis* spp. essential oils. The difference in activity compared to the other polyacetylenes mentioned might be associated to the absence in the *B. palustris* composition of appreciable quantities of polyacetylenic phenols or alcohols (as is the case for lobetyol and falcarinol for the named species) and the expressive presence of hydrocarbons (Table 1). Despite this oil was soluble in EtOH for conducting RSA tests, the transfer mechanism of a hydrogen atom from its main components to the DPPH (with the subsequent radical scavenging) could be prevented by the absence of structural motifs able to easily oxidize, such as phenolic or hydroxyl groups.

To date, many researchers have been conducted DPPH radical scavenging tests on *Baccharis* spp. essential oils. However, comparisons are difficult to conduct given the different approaches in the calculation of the activity. For example, Monteiro and coauthors (2022) [6] recently reported for *B. dracunculifolia* essential oil I (%) ranging from 49.40% to 50.82% (not mentioning the concentrations employed). Other

authors also reported RSA values conducting DPPH assays, not directly comparable with our results since different calculation formulas were used, *i.e.,* Souza and coauthors (2011) [48] for *B. tridentata* Vahl.; Tomazzoli and coauthors (2021) [8] and Luchesi and coauthors (2022) [4] for *B. dracunculifolia*; de Oliveira and coauthors (2019) [37] for *B. oreophila*, and Struiving and coauthors (2020) [49] for *B. trimera* and *B. myriocephala* DC. In all these reports, at least a marginal RSA was informed for the different *Baccharis* essential oils, being this (in the knowledge of the authors) for *B. palustris* the first study in which no activity was found.

CONCLUSION

In this contribution is presented the developing of TLC profiles for *B. palustris* essential oil using different visualization reagents, highlighting UV_{λ}= 365 nm and vanillin/H_3PO_4 as universal methods to detect polyacetylenic bands, and the useful application of NaDi to visualize the lachnophyllum acid methyl esters fraction. Applying HRGC/qMS, HRGC/TOF and GCxGC/TOF protocols, ten unreported terpenoids and a lachnophyllum lactone isomer (unknown stereochemistry) are informed for the first time in the species. In addition, six tentatively identified polyacetylenes and eighteen unknown components were also detected. These results highlighted the importance of GCxGC/TOF to identify hidden minor or trace components in complex samples as the studied in this work. Finally, *B. palustris* oil was an inactive DPPH radical scavenger. All the information obtained, suggests that we are only just at the beginning of understanding the polyacetylene specialized chemistry/biochemistry of *B. palustris*, and the genus *Baccharis* L. itself. In a broad frame, we conclude that our results may contribute to the preservation of this species as a valuable source of polyacetylenes.

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REFERENCES

1. Heiden G. *Baccharis*: Diversity and Distribution. In: Fernandes GW, Oki Y, Barbosa M; editors. *Baccharis*. From Evolutionary and Ecological Aspects to Social Uses and Medical Applications. Cham: Springer Nature; 2021. p. 23-80. https://doi.org/10.1007/978-3-030-83511-8_2
2. Manfron J, Raman V, Khan IA, Farago PV. Essential Oils of *Baccharis*: Chemical Composition and Biological Activities. In: Fernandes GW, Oki Y, Barbosa M; editors. *Baccharis*. From Evolutionary and Ecological Aspects to Social Uses and Medical Applications. Cham: Springer; 2021. p. 239-57. https://doi.org/10.1007/978-3-030-83511-8_10
3. Minteguiaga M, González HA, Ferreira F, Dellacassa E. *Baccharis dracunculifolia* DC. In: Máthé Á, Bandoni A; editors. Medicinal and Aromatic Plants of South America Vol. 2. Medicinal and Aromatic Plants of the World, vol 7. Cham: Springer; 2021. p. 85-105. https://doi.org/10.1007/978-3-030-62818-5_5
4. Luchesi LA, Paulus D, Busso C, Frata MT, Oliveira JB. Chemical composition, antifungal and antioxidant activity of essential oils from *Baccharis dracunculifolia* and *Pogostemon cablin* against *Fusarium graminearum*. Nat. Prod. Res. 2022;36(3):849-52. https://doi.org/10.1080/14786419.2020.1802267
5. Timbe PPR, da Motta ADS, Stincone P, Pinilla CMB, Brandelli A. Antimicrobial activity of *Baccharis dracunculifolia* DC and its synergistic interaction with nisin against food-related bacteria. J. Food. Sci. Technol. 2021;58:3010-8. https://doi.org/10.1007/s13197-020-04804-9
6. Monteiro EDS, Monteiro KDS, Montes PDS, da Camara CAG, Moraes MM, Fagg CW, et al. Chemical and antibacterial properties of *Baccharis dracunculifolia* DC essential oils from different regions of Brazil. J. Essent. Oil Res. 2022;34(6):524-32. https://doi.org/10.1080/10412905.2022.2103043
7. Cazella LN, de Oliveira HLDM, Bortolucci WDC, Rahal IL, Baretta IP, Gonçalves JE, et al. *Baccharis dracunculifolia* DC (Asteraceae) leaf and flower essential oils to control *Rhipicephalus microplus* Canestrini (Arachnida: Ixodidae) in the free-living stage. Res. Soc. Dev. 2020; 9(10):e5049108788. https://doi.org/10.33448/rsd-v9i10.8788

8. Tomazzoli MM, do Amaral W, Cipriano RR, Tomasi JDC, Gomes EN, Ferriani AP, et al. Chemical Composition and Antioxidant Activity of Essential Oils from Populations of *Baccharis dracunculifolia* DC. in Southern Brazil. Braz. Arch. Biol. Technol. 2021;64:e21190253. https://doi.org/10.1590/1678-4324-2021190253
9. Minteguiaga M, González A, Catalán CAN, Dellacassa E. Relationship between *Baccharis dracunculifolia* DC. and *B. microdonta* DC. (Asteraceae) by Their Different Seasonal Volatile Expression. Chem. Biodivers. 2021;18(6):e2100064. https://doi.org/10.1002/cbdv.202100064
10. Minteguiaga M, Catalán CAN, Cassel E, Dellacassa E. The “Other Vassoura Oil” and Volatile Fractions from *Baccharis uncinella* DC. (Asteraceae) as Potential Sources for Flavor and Fragrance Industry. In: Singh S; editor. Volatile Oils. Production, Composition and Uses. Biochemistry Research Trends. New York: Nova Science Publishers; 2021. p. 187-246. https://doi.org/10.52305/IZIM9776
11. Minteguiaga M, Umpierrez N, González A, Dellacassa E, Catalán CAN. New C_9-polyacetylenes from the essential oil of the highly endangered species *Baccharis palustris* Heering (Asteraceae). Phytochem. Lett. 2022;48:106-13. https://doi.org/10.1016/j.phytol.2022.01.012
12. Wagner H, Bladt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Photographs by Rickl V. 2nd ed. Heidelberg: Springer; 1996. https://doi.org/10.1007/978-3-642-00574-9
13. École Polytechnique Fédérale de Lausanne (EPFL). TLC Visualization Reagents [Internet]. Lausanne: EPFL; 2018 [cited 2023 Jun 6]. Available from: https://www.epfl.ch/labs/lcso/wp-content/uploads/2018/06/TLC_Stains.pdf
14. Ibanez SI, Dötterl S, Anstett MC, Baudino S, Caissard JC, Gallet C, Després. The role of volatile organic compounds, morphology and pigments of globeflowers in the attraction of their specific pollinating flies. New Phytol. 2010;188(2):451-63. https://doi.org/10.1111/j.1469-8137.2010.03317.x
15. Minteguiaga M, Mercado MI, Ponessa G, Catalán CAN, Dellacassa E. Morphoanatomy and essential oil analysis of *Baccharis trimera* (Less.) DC. (Asteraceae) from Uruguay. Ind. Crops Prod. 2018;112:488-98. https://doi.org/10.1016/j.indcrop.2017.12.040
16. Minteguiaga M, Fariña, L, Cassel E, Fiedler S, Catalán CAN, Dellacassa E. Chemical compositions of essential oil from the aerial parts of male and female plants of *Baccharis tridentata* Vahl. (Asteraceae). J. Essent. Oil Res. 2021;33(3):299-307. https://doi.org/10.1080/10412905.2020.1829720
17. International Organization for Standarization (ISO). International Standard 7609. Essential Oils-Analysis by Gas Chromatography on capillary columns-General Method. 1st ed. Geneva: ISO; 1985. https://www.iso.org/standard/14397.html
18. McLafferty FW. Wiley-NIST Registry/Mass Spectral Library. 9th Ed. [DVD-ROM]. Hoboken: John Wiley & Sons; 2008.
19. Mondello L. Flavors and Fragrances of Natural and Synthetic Compounds/Mass Spectral Library. Version 1.3. [CD-ROM]. Hoboken: John Wiley & Sons; 2008.
20. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4.1 edition. Carol Stream: Allured Publ; 2017. Available at: https://diabloanalytical.com/ms-software/essentialoilcomponentsbygcms/
21. Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 1990;503:1-24. https://doi.org/10.1016/S0021-9673(01)81487-4
22. Linstrom PJ, Mallard WG. NIST Chemistry WebBook. NIST Standard Reference Database Number 69 [Internet]. Gaithersburg: National Institute of Standards and Technology; c. 2022, [cited 2023 Jan 18]. Available from: https://doi.org/10.18434/T4D303
23. Koleva II, van Beek TA, Linssenn JPH, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 2002;13(1):8-17. https://doi.org/10.1002/pca.611
24. Razavi SM. Chemical Composition and Some Allelopathic Aspects of Essential Oils of (*Prangos ferulacea* L.) Lindl at Different Stages of Growth. J. Agric. Sci. Technol. 2012;14(2):349-56. http://jast.modares.ac.ir/article-23-9727-en.html
25. Minteguiaga M, González A, Cassel E, Umpierrez N, Fariña L. Dellacassa E. Volatile Constituents from *Baccharis* spp. L. (Asteraceae): Chemical Support for the Conservation of Threatened Species in Uruguay. Chem. Biodivers. 2018;15(5):e1800017. https://doi.org/10.1002/cbdv.201800017
26. Picman AK, Ranieri RL, Towers GHN, Lam J. Visualization reagents for sesquitterpene lactones and polyacetylenes on thin-layer chromatograms. J. Chromatogr. A 1980;189(2):187-98. https://doi.org/10.1016/S0021-9673(00)81518-6
27. Takamatsu H, Obayashi K. A Revised Method for Histochemical Demonstration of Cytochrome Oxidase. Acta Tuberc. Jpn. 1954;4(2):51-4. http://hdl.handle.net/2433/51760
28. Budel JM, Wang M, Raman V, Zhao J, Khan SI, Rehman JU, Techen N, Tekwani B, Monteiro LM, Heiden G, Takeda IJM, Farago PV, Khan IA. Essential Oils of Five *Baccharis* Species: Investigations on the Chemical Composition and Biological Activities. Molecules 2018;23(10):2620. https://doi.org/10.3390/molecules23102620
29. Kurdelas RR, López S, Lima B, Feresin GE, Zygadlo J, Zacchino S, López ML, Tapia A, Freile ML. Chemical composition, anti-insect and antimicrobial activity of *Baccharis darwinii* essential oil from Argentina, Patagonia. Ind. Crops Prod. 2012;40:261-7. https://doi.org/10.1016/j.indcrop.2012.03.024

30. Castillejos-Ramírez E, Pérez-Vásquez A, Torres-Colín R, Navarrete A, Andrade-Cetto A, Mata R. Antinociceptive Effect of an Aqueous Extract and Essential Oil from *Baccharis heterophylla*. Plants 2021;10(1):116. https://doi.org/10.3390/plants10010116
31. Albuquerque MRJR, Souza EB, Lins MUDS, Nogueira NAP, Lemos TLG, Silveira ER, Pessoa ODL. Composition and antimicrobial activity of the essential oil from aerial parts of *Baccharis trinervis* (Lam.) Pers. Arkivoc 2004;2004(6):59-65. https://doi.org/10.3998/ark.5550190.0005.608
32. Loayza I, Abujder D, Aranda R, Jakupovic J, Collin G, Deslauriers H, Jean FI. Essential oils of *Baccharis salicifolia*, *B. latifolia* and *B. dracunculifolia*. Phytochemistry 1995;38(2):381-9. https://doi.org/10.1016/0031-9422(94)00628-7
33. Silva ML, Costa-Silva TA, Antar GM, Tempone AG, Lago JHG. Chemical Constituents from Aerial Parts of *Baccharis sphenophylla* and Effects against Intracellular Forms of *Trypanosoma cruzi*. Chem. Biodivers. 2021;18(10):e2100466. https://doi.org/10.1002/cbdv.202100466
34. Minteguiaga M, Umpiérrez N, Fariña L, Falcão MA, Xavier VB, Cassel E, Dellacassa E. Impact of gas chromatography and mass spectrometry combined with gas chromatography and olfactometry for the sex differentiation of *Baccharis articulata* by the analysis of volatile compounds. J. Sep. Sci. 2015;38(17):3038-46. https://doi.org/10.1002/jssc.201500131
35. Weyerstahl P, Christiansen C, Marschall H. Constituents of Brazilian Vassoura oil. Flav. Fragr. J. 1996;11(1):15-23. https://doi.org/10.1002/(SICI)1099-1026(199601)11:1<15::AID-FFJ541>3.0.CO;2-H
36. Chaverri C, Cicció JF. Essential oils of *Baccharis trinervis* (Asteraceae) from Costa Rica. Rev. Biol. Trop. 2017;65(4):1307-21. https://doi.org/10.15517/rbt.v65i4.27845
37. de Oliveira CT, Maia BHLDNS, Ferriani AP, Santos VAQ, da Cunha MAA, Teixeira SD. Chemical Characterization, Antioxidant Capacity and Antimicrobial Potential of Essential Oil from the Leaves of *Baccharis oreophila* MALME. Chem. Biodivers. 2019;16(2):e1800372. https://doi.org/10.1002/cbdv.201800372
38. Meshkatalsadat MH, Mirzaei HH. Chemical compositions of the Essential Oils of Stems, Leaves and Flowers of *Prangos acaulis* (Dc) Bornm. Pak. J. Biol. Sci. 2007;10(16):2775-7. https://doi.org/10.3923/pjbs.2007.2775.2777
39. Zhao M, Xiao L, Linghu KG, Zhao G, Chen Q, Shen L, Dar P, Chen M, Hu Y, Zhang J. Yu H. Comprehensive comparison on the anti-inflammation and GC-MS-based metabolomics discrimination between *Bupleuri chinense* DC. and *B. scorzonerifolium* Willd. Front. Pharmacol. 2022;13:1005011. https://doi.org/10.3389/fphar.2022.1005011
40. Minto RE, Blacklock BJ. Biosynthesis and function of polyacetylenes and allied natural products. Prog. Lipid. Res. 2008,47(4):233-306. https://doi.org/10.1016/j.plipres.2008.02.002
41. Rivera AP, Arancibia L, Castillo M. Clerodane Diterpenoids and Acetylenic Lactones from *Baccharis paniculata*. J. Nat. Prod. 1989;52(2):433-5. https://doi.org/10.1021/np50062a045
42. Fernández-Aparicio M, Soriano G, Masi M, Carretero P, Vilariño-Rodríguez S, Cimmino A. (4*Z*)-Lachnophyllum Lactone, an Acetylenic Furanone from *Conyza bonariensis*, Identified for the First Time with Allelopathic Activity against *Cuscuta campestris*. Agriculture 2022;12(6):790. https://doi.org/10.3390/agriculture12060790
43. Queiroz SCN, Cantrell CL, Duke SO, Wedge DE, Nandula VK, Moraes RM, Cerdeira AL. Bioassay-Directed Isolation and Identification of Phytotoxic and Fungitoxic Acetylenes from *Conyza canadensis*. J. Agric. Food Chem. 2012;60(23):5893-8. https://doi.org/10.1021/jf3010367
44. Nawamaki T, Sakakibara T, Ohta K. Isolation and Identification of Lachnophyllum Lactone and Osthol as Repellents against a Sea Snail. Agric. Biol. Chem. 1979;43(7):1603-4. https://doi.org/10.1080/00021369.1979.10863672
45. Matsunaga H, Katano M, Yamamoto H, Fujito H, Mori M, Takata K. Cytotoxic Activity of Polyacetylen Compounds in *Panax ginseng* C. A. MEYER. Chem. Pharm. Bull. 1990;38(12):3480-2. https://doi.org/10.1248/cpb.38.3480
46. Dumlu MU, Gurkan E, Tuzlaci E. Chemical composition and antioxidant activity of *Campanula alliariifolia*. Nat. Prod. Res. 2008;22(6):477-82. https://doi.org/10.1080/14786410701640429
47. Tel-Çayan G, Duru ME. Chemical characterization and antioxidant activity of *Eryngium pseudothoriifolium* and *E. thorifolium* essential oils. J. Res. Pharm. 2019;23(6):1106-14. https://doi.org/10.35333/jrp.2019.75
48. Souza SP, Cardoso MG, Souza PE, Guimarães LGL, Andrade J, Mallet ACT, et al. [*Baccharis tridentata* Vahl essential oil: chemical composition, and antioxidant and fungitoxic activities and morphological characterization of secretory structures by scanning electron microscopy]. Rev. Bras. Pl. Med. 2011;13(4):456-66. https://doi.org/10.1590/S1516-05722011000400011
49. Struiving S, Hacke ACM, Simionatto EL, Scharf DR, Klimaczewski CV, Besten MA, et al. Effects of Gender and Geographical Origin on the Chemical Composition and Antiradical Activity of *Baccharis myriocephala* and *Baccharis trimera*. Foods 2020;9(10):1433. https://doi.org/10.3390/foods9101433