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Chemical Profiles and Cytotoxic Activities of Essential Oils from Six Species of *Baccharis* Subgenus *Coridifoliae* (Asteraceae)

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Several *Baccharis* species are popularly known in traditional medicine as *"carquejas"*, *"vassouras"*, *"ervas-santas"* and *"mio-mios"*, and are used as anti-inflammatories, digestives, and diuretics. This study aimed to investigate the chemical compositions and cytotoxic activities of essential oils (EOs) of six *Baccharis* species belonging to subgenus *Coridifoliae*, namely *B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea*, and *B. pluricapitulata*. GC/MS analyses of the EOs showed that the oxygenated sesquiterpenes spathulenol (7.32–38.22 %) and caryophyllene oxide (10.83–16.75 %) were the major components for all the species. The EOs of almost all species were cytotoxic against cancer (BT-549, KB, SK-MEL and SK-OV-3) and normal kidney (VERO and LLC-PK1) cell lines, whereas *B. erigeroides* EO showed cytotoxicity only against LLC-PK1. This article augments the current knowledge about the chemical-biological properties of *Baccharis* subgenus *Coridifoliae* and discusses the therapeutic potentials of these economically unexploited plants.

Introduction

The genus *Baccharis* L. (Asteraceae) is widespread in the Americas. It comprises about 442 species, 185 of them occurring in Brazil.[1] In Latin America, different *Baccharis* species are commonly called (in Portuguese or in Spanish) *"carquejas"* (plants with cladodes), *"vassouras"*, "ervas-santas" and "mio-mio" (plants with regular leaves and stems),[2] being used in folk medicine as anti-inflammatories, digestives, and diuretics.[3] The medicinal properties of *Baccharis* species are attributed to chemical compounds usually found in the plants, which include: essential oils (EOs, rich in volatile mono- and sesquiterpenes), and no volatile (or semi-volatile) flavonoids, phenolic acids, di- and triterpenes.[4,5]

Baccharis is currently divided into seven subgenera: *Baccharis*, *Coridifoliae* (DC.) G.Heiden, *Heterothalamulopsis* (Deble, A.S.Oliveira & Marchiori) G.Heiden, *Heterothalamus* (Less.) G.Heiden, *Molina* (Pers.) Heering, *Oblongifoliae* (DC.) G.Heiden, and *Tarchonanthoides* Heering.[1] The subgenus *Coridifoliae* comprises ten species, namely *B. albilanosa* A.S.Oliveira & Deble, *B. artemisioides* Hook. & Arn., *B. bicolor* (Joch.Müll.) G.Heiden, *B. coridifolia* DC., *B. erigeroides* DC., *B. napaea* G.Heiden, *B. ochracea* Spreng., *B. pluricapitulata* (Deble) G.Heiden, *B. scabrifolia* G.Heiden, and *B. suberectifolia* A.S.Oliveira & Deble.[1] From

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them, *B. coridifolia* is frequently cited as toxic, mainly for cattle, due to the occurrence of macrocyclic trichothecenes in its aerial parts.[6] However, a beneficial biological activity as an antimicrobial has been reported for the EO of this species, including a synergistic effect when combined with some commercial antibiotics such as cephalothin and tetracycline.[5,7–9] In the aerial parts of *B. ochracea*, terpenes (di- and tri- derivatives) and phenolic compounds (chlorogenic and isochlorogenic acid and isoquercitrin) have been identified,[10] which presumably are responsible for the cytotoxicity of its organic and aqueous extracts.[11] Moreover, *B. ochracea* essential oil demonstrated antifungal activity (100% mycelial growth inhibition) against the phytopathogen *Alternaria alternata*, thus demonstrating the potential to be considered a bio-fungicide.[12] Detailed studies on *B. albilanosa*, *B. erigeroides*, *B. napaea*, and *B. pluricapitulata* are scarce: only a morphoanatomical study has been published,[13] and neither chemical nor biological activity/pharmacological research has been conducted so far.

Considering that *Baccharis* species are widely used in traditional medicine, have a high socioeconomic value, therapeutic potential and limited information related to chemical and pharmacological studies, in addition to these important aspects for new studies, this article aimed to present novelties about the EO chemical profiles and evaluate the cytotoxic potential of six species of *Baccharis* (*B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea*, and *B. pluricapitulata*). Furthermore, it presents for the first time the EO chemical profile and cytotoxic potential of *B. albilanosa*, *B. erigeroides*, *B. napaea* and *B. pluricapitulata.*

Results and Discussion

Chemical Profile of Essential Oils (EOs)

Baccharis species produce EOs containing mainly volatile monoterpenes and sesquiterpenes (hydrocarbons or oxygenated) (Figure 1), with the latter class being the most abundant in most species.[14] The biplot graph showed that Boch and Balb produce high concentrations of oxygenated sesquiterpenes (OS). Bplu and Beri express high production of sesquiterpene hydrocarbon (SH), while Bnap and Bcor the major contribution for the variance is monoterpene hydrocarbon (MH) and oxygenated monoterpene (OM). EOs extracted by hydrodistillation from *Baccharis* species are clear or colored, with a strong and characteristic aroma, and generally have a lower density than water.[5]

In this work, EOs were extracted by hydrodistillation from the aerial parts (leaves and stems) of *B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea* and *B. pluricapitulata.* The EOs presented a strong and characteristic aroma, slightly yellowish color, and density lower than that of water. The EOs yields (*v/w*) were as follows: *B. albilanosa* (0.03%), *B. coridifolia* (0.06%), *B. erigeroides* (0.04%), *B. napaea* (0.04%), *B. ochracea*

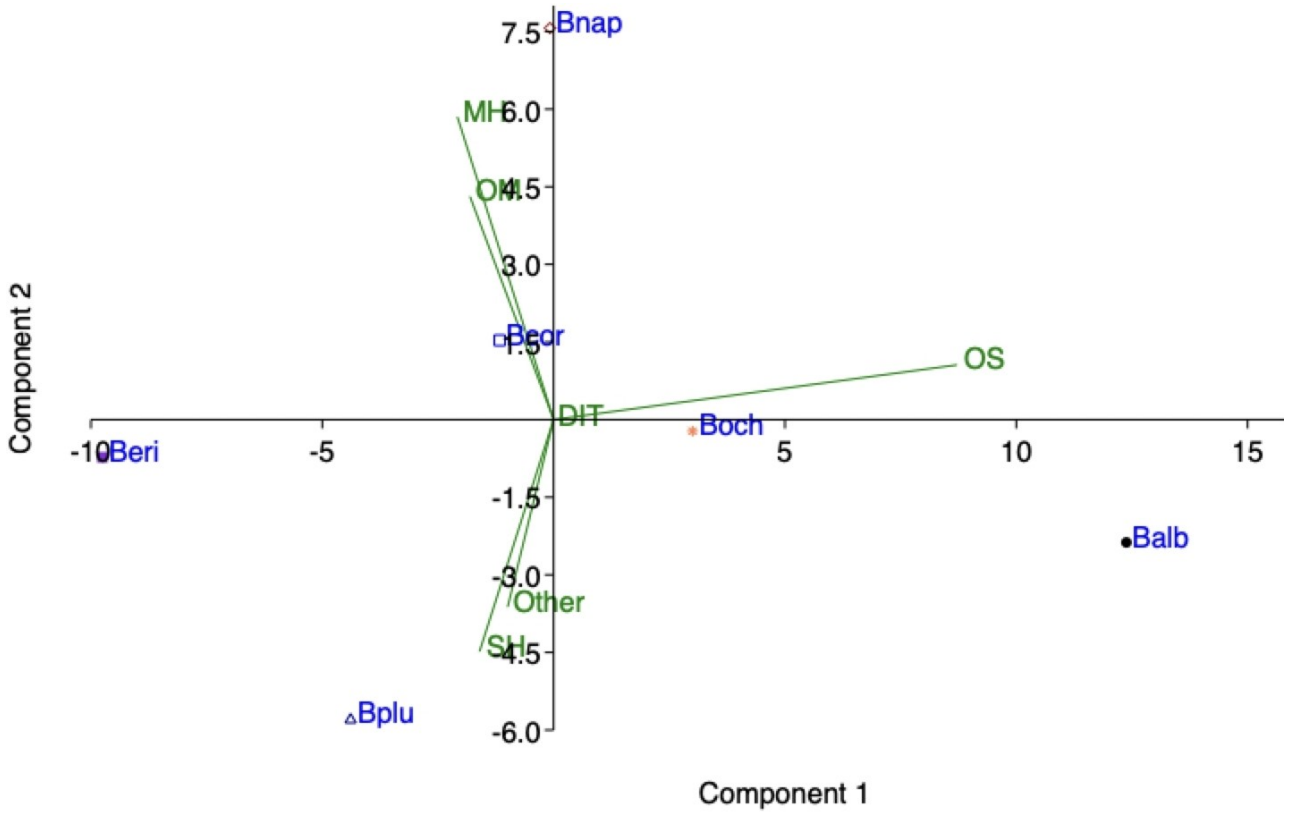

Figure 1. Biplot graph of principal component analysis based on six essential oil samples from *Baccharis* genus, and four chemical classes (loading) obtained from chromatographic analyses (GC/MS). Balb = *B. albilanosa*, Bcor = *B. coridifolia*, Beri = *B. erigeroides*, Bnap = *B. napaea*, Boch = *B. ochracea* and Bplu = *B. pluricapitulata.*

(0.04%), and *B. pluricapitulata* (0.03%). Previous reports in the literature inform that the EOs yields for *Baccharis* species usually ranged between 0.08–2.82%.[5] However, *B. anomala* DC. and *B. dentata* (Vell.) G.M.Barroso (among others) exhibited low yield levels (around 0.03–0.05%), as reported previously by Xavier et al. (2013, 2017).[15,16] The production of EOs by plants can be influenced by physiological and genetic variations particular to the plant species, phenological factors, seasonality, and environmental conditions.[17] In fact, among other studies, Xavier et al. (2017)[16] demonstrated the EO yield and composition of *B. dentata* and *B. uncinella* DC. varied between summer and autumn collections. Moreover, Tomazzoli et al. (2021)[18] reported variation in the EO yield, composition, and antiradical activity (DPPH) across ten different *B. dracunculifolia* DC. populations from the Brazilian Paraná State. In addition, the drying conditions applied to the plant material, the grinding process, the storage conditions, and the extraction methods used to obtain the EOs can influence the yield obtained.[19]

The EO chemical profiles of the six species of *Baccharis* subgenus *Coridifoliae* were investigated and are compared in Table 1. Furthermore, the identification of the peaks for these six species with abundance $\geq$0.5% for at least one species is shown in Figure 2. The principal component analysis showed the dispersion of three distinct groups (Figure 3). On the axis of principal component **1**, representing 87% of variance show α-pinene (**1**) and β-pinene (**2**) as the major compounds for the contribution to the formation of the groups (Bcor, Beri, and Bnap). On the axis of principal component **2**, representing 7% of the variance, the compound Caryophyllene oxide (**3**) contributes more to the dispersion of Bplu and spathulenol (**4**) to the dispersion of Boch and Balb (Figure 4).

As discussed above, EOs of *Baccharis* species mainly comprise monoterpenes and sesquiterpenes (hydrocarbons and oxygenated derivatives), the latter being more abundant.[5] In the present study, higher levels of sesquiterpenes (52.02–71.04%) were found in all the species studied. They comprised 3.83–11.91% of hydrocarbons and 42.74–63.57% of oxygenated derivatives. Monoterpenes (1.06–17.76%) were also found in all the studied species and comprised 0.41–9.52% of hydrocarbons and 0.65–8.25% of oxygenated derivatives. Furthermore, it was possible to identify two diterpenes: neophytadiene which was present in all the species, and manool oxide tentatively identified in *B. albilanosa*, *B. coridifolia* and *B. ochracea*. Previous studies reported neophytadiene in the EO of *B. trimera* (Less.) DC. and *B. uncinella* DC.[20–22] As far as we know, manool oxide is tentatively identified (without absolute confirmation with a pure standard) in this study for the first time in the *Baccharis* genus. But the putative precursor, *ent*-manool has been previously reported to be present in the roots of *B. oxyodonta* DC.[23] In addition, 2.50–8.03% of the EOs composition was found to be represented by other aliphatic and phenylpropanoid type compounds presenting aldehyde, alcohol, carboxyl, ether, and ketone functions.

Previous studies have shown that the main constituents of *Baccharis* EOs were a) monoterpenes: α-thujene, β-pinene, camphor, limonene, sabinene, and thymol, and b) sesquiterpenes: β-caryophyllene, spathulenol, nerolidol, and caryophyllene oxide.[5] In the present study, the EOs from the six species of *Baccharis* subgenus *Coridifoliae* analyzed had spathulenol (7.48–38.56%) and caryophyllene oxide (11.04–17.11%) as the main constituents. Other constituents such as: hexanal, *trans*-2-hexenal, *trans*-3-hexenol, *n*-hexanol, benzaldehyde, α-pinene, 6-

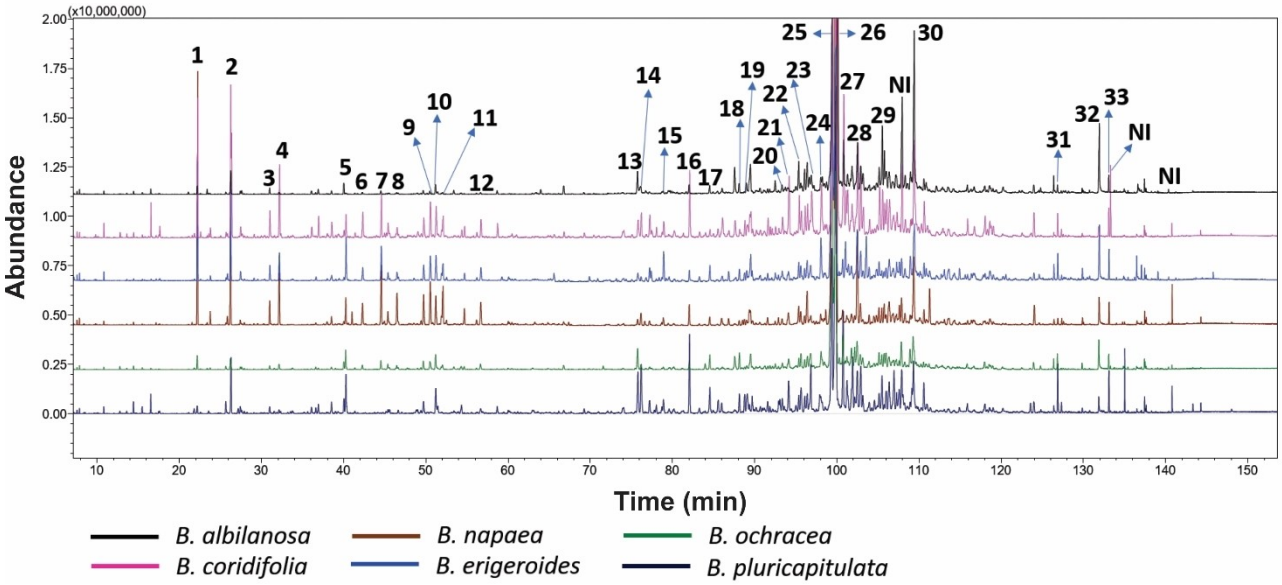

Figure 2. Peak identification for six species of *Baccharis* subgenus *Coridifoliae* (Asteraceae) after GC/MS analyses (abundance $\geq$0.5% for at least one species). **1**. α-pinene, **2**. β-pinene, **3**. *p*-cymene, **4**. limonene, **5**. linalool, **6**. α-campholenal, **7**. *trans*-pinocarveol, **8**. pinocarvone, **9**. myrtenal, **10**. α-terpineol, **11**. myrtenol, **12**. carvone, **13**. *trans*-β-damascenone, **14**. cyclosativene, **15**. β-elemene, **16**. *trans*-β-caryophyllene, **17**. neryl acetone, **18**. *trans*-β-ionone, **19**. germacrene D, **20**. *trans*-calamenene, **21**. δ-cadinene, **22**. α-calacorene, **23**. salvadienol, **24**. mint oxide, **25**. spathulenol, **26**. caryophyllene oxide, **27**. salvial-4(14)-en-1-one, **28**. humulene epoxide II, **29**. caryophylla-4(12),8(13)-dien-5α-ol, **30**. cadalene, **31**. hexahydrofarnesyl acetone, **32**. palmitic acid, **33**. ethyl palmitate. **NI**. not identified. **Not tagged**: γ-muurolene, globulol, viridiflorol, cubeban-11-ol, guaiol, torilenol, junenol, 1,10-di-*epi*-cubenol, alismol, α-muurolol, β-eudesmol, α-cadinol, 7-*epi*-α-eudesmol, and bulnesol.

Table 1. Chemical profile of essential oils from six species of *Baccharis* subgenus *Coridifoliae* (Asteraceae).

No.	LRI_exp	LRI_lit	Compound	%					
				Balb	Bcor	Beri	Bnap	Boch	Bplu
1	795	801	Hexanal	0.03	0.08	0.09	0.04	0.04	0.08
2	837	846	*trans*-2-Hexenal	0.01	0.03	0.02	0.01	0.03	0.04
3	840	844	*trans*-3-Hexenol	0.03	0.04	0.03	0.03	0.07	0.18
4	851	858	1-Hexen-6-ol	nd	nd	nd	nd	nd	tr
5	857	863	*n*-Hexanol	0.02	0.03	0.02	0.01	0.04	0.10
6	880	889	2-Heptanone	nd	0.01	nd	nd	nd	0.01
7	892	901	Heptanal	0.01	0.03	0.02	nd	0.01	0.03
8	919	921	Tricyclene	0.03	nd	nd	0.01	nd	nd
9	923	924	α-Thujene	nd	0.03	0.02	0.04	nd	nd
10	942	952	Benzaldehyde	0.01	0.05	0.03	0.01	0.05	0.10
11	930	932	α-pinene	0.10	1.44	2.09	4.37	0.38	0.16
12	946	954	6-Methyl-2-heptanone	0.01	0.06	0.03	nd	0.02	0.02
13	943	946	Camphene	0.07	0.02	0.03	0.04	0.04	nd
14	949	953	Thuja-2.4(10)-diene	0.01	0.10	0.09	0.23	0.05	nd
15	956	959	*n*-Heptanol	nd	nd	nd	nd	nd	0.01
16	974	981	6-Methyl-5-hepten-2-one (sulcatone)	0.04	0.06	0.06	0.03	0.12	0.27
17	966	969	Sabinene	nd	0.05	0.18	0.16	nd	nd
18	971	974	β-pinene	nd	1.92	2.09	2.68	0.47	1.25
19	983	988	Dehydro-1,8-cineole	nd	0.03	nd	0.02	nd	nd
20	981	984	2-Pentyl-furan	0.04	0.12	0.22	0.09	0.07	0.14
21	991	998	Octanal	0.06	0.07	nd	0.03	0.08	0.08
22	985	988	Myrcene + Ethyl hexanoate (Ethyl caproate)	tr	0.02	0.04	0.04	0.04	0.05
23	1025	1036	Benzene acetaldehyde	nd	nd	nd	0.01	nd	nd
24	1007	1008	δ-3-Carene	nd	0.02	nd	nd	nd	nd
25	1016	1020	*p*-Cymene	0.10	0.34	0.33	0.48	0.14	0.16
26	1022	1025	β-Phellandrene	nd	0.02	nd	0.01	nd	0.03
27	1022	1024	Limonene	0.03	0.90	0.56	1.16	0.17	0.08
28	1042	1049	*trans*-2-Octenal	0.01	0.02	0.03	0.01	0.02	0.05
29	1061	1077	*p*-Tolualdehyde	0.02	0.05	0.07	nd	0.07	0.09
30	1051	1054	γ-Terpinene	nd	0.04	nd	0.02	0.02	0.04
31	1061	1065	*n*-Octanol + *cis*-Linalool oxide (furanoid)	0.02	0.03	0.10	0.04	0.09	0.15
32	1082	1089	*p*-Cymenene	0.06	0.22	0.18	0.20	0.10	0.31
33	1082	1086	Terpinolene	nd	0.10	nd	0.04	0.03	0.07
34	1091	1099	α-Pinene oxide	nd	nd	0.06	nd	nd	nd
35	1093	1100	Nonanal	0.17	0.10	0.07	0.08	0.26	0.38
36	1090	1095	Linalool	0.02	0.32	1.05	0.60	0.70	1.10
37	1083	1084	Hotrienol	0.01	nd	nd	nd	nd	nd
38	1096	1102	Perillene	nd	0.01	0.02	0.04	nd	nd
39	1090	1091	1-Undecene	nd	nd	nd	0.26	nd	nd
40	1107	1114	*endo*-Fenchol	nd	0.01	nd	0.02	nd	nd
41	1114	1122	α-Campholenal	0.03	0.39	0.33	0.53	0.15	nd
42	1112	1118	*cis*-*p*-Menth-2-en-1-ol	nd	0.02	nd	0.03	nd	0.02
43	1125	1133	*cis*-*p*-Mentha-2,8-dien-1-ol	nd	0.03	0.02	0.03	nd	nd
44	1128	1135	*trans*-Pinocarveol	0.06	0.63	0.88	1.41	0.34	nd
45	1131	1137	*cis*-Verbenol	nd	0.07	nd	0.13	nd	nd
46	1134	1140	*trans*-Verbenol	0.02	0.22	0.23	0.37	0.23	0.10
47	1148	1158	*trans*-Pinocamphone	nd	0.04	nd	nd	nd	nd

Table 1. continued

No.	LRI_exp	LRI_lit	Compound	%					
				Balb	Bcor	Beri	Bnap	Boch	Bplu
48	1150	1160	Pinocarvone	0.04	0.14	0.25	0.80	0.19	nd
49	1150	1159	2-*trans*-nonenal	0.03	nd	nd	nd	0.07	0.08
50	1157	1166	*p*-Mentha-1,5-dien-8-ol	nd	0.04	nd	nd	0.05	nd
51	1157	1165	Borneol	nd	0.03	0.01	0.04	0.03	nd
52	1168	1179	*p*-Methylacetophenone (Melilotal)	0.02	0.06	0.07	0.06	nd	0.02
53	1169	1177	*p*-Cymen-8-ol + Terpinen-4-ol	nd	nd	nd	nd	0.36	0.40
54	1164	1167	Octanoic acid (Caprylic acid)	0.02	nd	0.02	nd	nd	0.03
55	1183	1195	Myrtenal	0.06	0.54	0.65	1.05	0.41	0.04
56	1179	1187	*trans-p*-Mentha-1(7),8-dien-2-ol	nd	0.06	0.04	0.06	0.04	nd
57	1179	1186	α-Terpineol	0.18	0.47	0.62	0.68	0.66	0.68
58	1193	1204	Verbenone	nd	0.10	0.15	0.19	0.09	0.02
59	1186	1194	Myrtenol	0.04	0.32	0.44	0.93	0.17	nd
60	1190	1196	Ethyl octanoate (ethyl caprylate)	tr	0.01	nd	nd	0.03	0.03
61	1195	1201	Decanal	0.06	0.03	0.02	nd	0.14	0.08
62	1214	1226	*cis*-Carveol	0.01	0.18	0.21	0.39	0.12	0.02
63	1226	1238	Cumin aldehyde	0.02	0.08	0.09	0.12	0.07	0.05
64	1228	1239	Carvone	0.03	0.27	0.36	0.54	0.23	0.15
65	1226	1235	Neral	nd	nd	0.03	nd	nd	nd
66	1233	1244	Carvotanacetone	nd	0.02	nd	nd	nd	0.03
67	1243	1249	Geraniol	nd	0.02	0.10	0.08	0.05	0.06
68	1258	1269	Perilla aldehyde	nd	0.09	nd	0.07	0.05	nd
69	1255	1264	Geranial	nd	nd	0.07	-	0.04	0.03
70	1254	1261	*cis*-Chrysanthenyl acetate	nd	0.06	nd	nd	nd	nd
71	1271	1283	α-Terpinen-7-al	nd	0.01	nd	nd	nd	nd
72	1262	1266	*n*-Decanol	0.03	nd	nd	nd	nd	nd
73	1263	1267	Nonanoic acid (pelargonic acid)	0.05	nd	0.04	nd	0.07	0.12
74	1278	1289	*p*-Cymen-7-ol	nd	0.02	0.04	0.09	nd	nd
75	1277	1284	Bornyl acetate	0.09	nd	nd	nd	nd	nd
76	1286	1294	Perilla alcohol	nd	0.03	0.04	0.06	0.06	nd
77	1285	1290	Dehydroedulan I	nd	0.06	0.22	0.06	nd	nd
78	1290	1298	Carvacrol	nd	nd	nd	nd	0.04	nd
79	1289	1294	Ethyl nonanoate (Ethyl pelargonate)	0.01	0.02	0.03	0.02	0.03	0.05
80	1304	1315	*trans,trans*-2,4-Decadienal	nd	nd	0.06	0.08	nd	0.10
81	1298	1305	Undecanal	0.15	nd	nd	nd	nd	nd
82	1289	1290	1-Tridecene	nd	nd	nd	0.09	nd	nd
83	1303	1309	Edulan I	nd	nd	nd	0.04	nd	0.07
84	1323	1326	Silphiperfol-5-ene	nd	nd	0.08	nd	nd	nd
85	1345	1356	Eugenol	nd	nd	nd	nd	0.06	nd
86	1341	1349	Dehydro-ar-ionene (1,1,6-Trimethyl-1,2-dihydro-naphthalene = TDN)	nd	nd	0.03	nd	nd	0.07
87	1341	1345	7-*epi*-Silphiperfol-5-ene	nd	nd	0.09	nd	nd	nd
88	1352	1361	*cis*-β-Damascenone	nd	nd	nd	nd	nd	0.02
89	1344	1345	α-Cubebene	0.05	0.22	0.10	0.08	0.04	0.32
90	1353	1358	Silphiperfola-4,7(14)-diene	nd	nd	0.08	nd	nd	nd
91	1360	1364	Decanoic acid (Capric acid)	nd	nd	0.05	0.02	nd	0.06
92	1374	1383	*trans*-β-Damascenone	0.46	0.35	0.31	0.17	0.87	1.27
93	1365	1369	Cyclosativene	0.28	0.41	0.15	0.33	0.23	1.63
94	1369	1373	α-Ylangene	nd	nd	nd	0.12	nd	0.02

Table 1. continued

No.	LRI_exp	LRI_lit	Compound	%					
				Balb	Bcor	Beri	Bnap	Boch	Bplu
95	1371	1374	α-Copaene	0.03	0.37	0.36	0.12	0.12	0.37
96	1377	1382	Modheph-2-ene	nd	0.15	0.28	0.02	0.03	nd
97	1373	1374	Isoledene	nd	nd	nd	nd	nd	0.09
98	1382	1387	β-Bourbonene	nd	0.16	0.08	0.02	0.03	0.23
99	1386	1390	Sativene	0.03	0.09	0.11	0.08	0.05	0.18
100	1386	1389	β-Elemene	0.06	0.25	0.80	0.19	0.10	0.42
101	1401	1409	2,6-Dimethylnaphthalene	0.08	0.07	nd	nd	nd	nd
102	1400	1408	Dodecanal	0.06	0.03	nd	nd	0.06	nd
103	1396	1400	Ylanga-2,4(15)-diene	0.05	0.20	0.11	0.09	0.15	0.23
104	1405	1409	α-Gurjunene	0.04	0.08	0.09	0.01	0.02	0.09
105	1413	1417	*trans*-Caryophyllene	0.19	1.07	0.31	0.52	0.19	2.35
106	1425	1430	β-Copaene	0.03	0.09	0.15	0.05	0.04	0.17
107	1427	1431	β-Gurjunene (Calarene)	nd	nd	nd	nd	nd	0.04
108	1431	1434	Neryl acetone	0.17	0.15	0.51	0.22	0.63	0.91
109	1449	1457	*allo*-Aromadendrene-4(15),10(14)-diene	0.08	0.16	nd	nd	0.07	0.45
110	1447	1452	α-Humulene	nd	nd	nd	0.28	nd	0.36
111	1453	1458	*allo*-Aromadendrene	0.03	0.10	0.30	0.18	0.03	0.05
112	1476	1487	*trans*-β-Ionone	0.22	0.12	0.42	0.12	0.67	0.54
113	1469	1475	γ-Gurjunene	nd	0.07	nd	nd	0.11	nd
114	1469	1475	*trans*-Cadina-1(6),4-diene	0.05	0.04	nd	0.13	nd	0.04
115	1472	1478	γ-Muurolene	0.04	0.26	0.25	0.14	0.10	0.52
116	1477	1484	Germacrene D	0.27	0.33	0.31	0.13	0.19	0.82
117	1477	1483	α-Amorphene	nd	nd	0.13	nd	nd	nd
118	1482	1489	β-Selinene	0.07	0.15	0.30	0.16	0.15	0.11
119	1489	1496	Valencene	nd	nd	0.32	0.10	nd	0.24
120	1491	1498	α-Selinene	nd	nd	0.06	tr	nd	0.14
121	1495	1500	α-Muurolene	0.10	0.30	0.22	0.18	0.15	0.36
122	1502	1509	Tridecanal	0.05	0.18	0.14	0.02	nd	nd
123	1507	1513	γ-Cadinene	0.10	0.20	0.21	0.20	0.15	0.38
124	1513	1521	*trans*-Calamenene	0.26	0.44	0.38	0.21	0.32	0.49
125	1516	1522	δ-Cadinene	0.17	1.14	0.33	0.48	0.83	1.09
126	1525	1534	Liguloxide	nd	nd	0.12	nd	nd	nd
127	1535	1544	α-Calacorene	0.77	0.75	0.61	0.70	0.55	0.58
128	1540	1545	Salviadienol	0.66	1.15	0.80	0.47	1.25	1.88
129	1554	1564	β-Calacorene	0.23	0.18	0.27	0.14	0.17	0.14
130	1551	1557	Mint oxide	0.39	1.22	1.60	0.33	0.90	0.46
131	1561	1570	Caryophyllenyl alcohol	0.34	0.31	0.24	0.22	0.38	0.33
132	1555	1561	*trans*-Nerolidol	nd	nd	0.38	nd	nd	nd
133	1560	1567	Palustrol	0.30	0.25	nd	0.41	0.27	nd
134	1569	1577	Spathulenol	38.56	25.05	19.65	28.47	28.56	7.48
135	1574	1582	Caryophyllene oxide	14.03	11.30	11.04	15.18	12.73	17.11
136	1580	1590	Globulol	0.53	0.25	0.32	0.35	0.23	0.18
137	1584	1594	Salvial-4(14)-en-1-one	1.21	3.08	0.81	0.64	1.67	3.68
138	1584	1592	Viridiflorol	0.27	0.88	1.29	0.11	0.45	1.54
139	1586	1595	Cubeban-11-ol	nd	nd	0.59	nd	nd	nd
140	1591	1600	Guaiol	nd	nd	nd	nd	nd	2.48
141	1598	1608	Humulene epoxide II	1.69	2.46	1.87	2.87	1.65	1.51

Table 1. continued

No.	LRI_exp	LRI_lit	Compound	%					
				Balb	Bcor	Beri	Bnap	Boch	Bplu
142	1597	1604	Torilenol	0.80	1.26	1.16	0.72	0.87	1.96
143	1604	1611	Tetradecanal	nd	nd	nd	nd	0.16	nd
144	1609	1618	Junenol	nd	nd	0.56	nd	0.37	0.58
145	1613	1618	1,10-di-*epi*-Cubenol	0.70	1.03	0.67	0.56	0.85	0.71
146	1628	1639	Caryophylla-4(12),8(13)-dien-5α-ol	1.63	0.86	0.34	0.51	0.68	1.44
147	1615	1619	Alismol	0.97	0.60	0.34	0.64	0.71	0.27
148	1634	1644	α-Muurolol	0.87	1.01	1.07	1.06	1.04	1.50
149	1632	1640	*epi*-α-Muurolol	nd	nd	nd	nd	0.26	nd
150	1636	1646	Agarospirol	0.28	nd	nd	nd	nd	nd
151	1639	1649	β-Eudesmol	nd	nd	nd	nd	0.64	1.94
152	1643	1652	α-Cadinol	0.22	nd	0.63	0.73	0.44	1.09
153	1651	1662	7-*epi*-α-Eudesmol	nd	nd	nd	nd	nd	0.50
154	1659	1670	Bulnesol	nd	nd	nd	nd	nd	0.77
155	1664	1675	Cadalene	4.52	nd	2.79	nd	nd	nd
156	1669	1674	β-Bisabolol	nd	nd	nd	nd	nd	0.27
157	1706	1713	Pentadecanal	nd	nd	0.42	nd	0.08	0.21
158	1756	1761	Tetradecanoic acid (Myristic acid)	0.24	nd	0.19	nd	0.22	0.27
159	1792	1803	14-Hydroxy-δ-cadinene	0.11	0.07	0.05	0.04	0.10	0.07
160	1791	1795	Ethyl tetradecanoate (Ethyl myristate)	0.04	nd	nd	0.04	0.07	0.27
161	1805	1810	Hexadecanal	0.10	0.03	0.07	nd	0.09	0.08
162	1837	1840	Hexahydrofarnesyl acetone	0.13	0.24	0.51	0.12	0.37	0.86
163	1843	1844	Neophytadiene	0.04	0.07	0.11	0.09	0.03	0.19
164	1859	1865	Pentadecanoic acid	0.08	nd	0.12	nd	nd	nd
165	1887	1890	Ethyl pentadecanoate	nd	tr	0.04	nd	nd	0.02
166	1889	1892	2-Heptadecanone	nd	nd	nd	nd	nd	0.03
167	1905	1913	*trans*,*trans*-5,9-Farnesyl acetone	0.05	0.03	0.17	0.09	0.12	0.08
168	1914	1922	Heptadecanal	nd	nd	nd	nd	nd	0.04
169	1916	1920	Methyl hexadecanoate (Methyl palmitate)	nd	nd	nd	nd	nd	0.02
170	1955	1960	Hexadecanoic acid (Palmitic acid)	1.18	0.13	1.22	0.53	0.88	0.36
171	1968	1975	Ethyl *trans*-9-hexadecenoate (Ethyl *trans*-9-palmitoleate)	nd	nd	nd	nd	nd	0.05
172	1982	1987	Manool oxide	0.04	0.01	nd	nd	0.03	nd
173	1988	1992	Ethyl hexadecanoate (Ethyl palmitate)	0.17	0.18	0.36	0.23	0.25	0.49
174	2016	2024	Octadecanal	nd	nd	nd	nd	0.03	nd
			Total identified (%)	76.06	70.08	70.09	78.43	69.39	72.74
			Monoterpenoids Hydrocarbon (%)	0.41	5.24	5.64	9.52	1.43	2.17
			Oxygenated Monoterpenoids (%)	0.65	4.28	5.77	8.25	4.18	2.86
			Sesquiterpenoids Hydrocarbon (%)	7.46	7.22	9.28	4.68	3.83	11.91
			Oxygenated Sesquiterpenoids (%)	63.57	50.77	42.74	53.31	54.07	47.78
			Diterpenes (%)	0.08	0.08	0.11	0.09	0.06	0.19
			Others (%)	3.91	2.53	5.89	2.50	5.94	8.03

LRI_exp: linear retention index obtained experimentally in this research; LRI lit: linear retention index obtained from the literature;[31,32] nd: not detected; tr: trace (< 0.01 %) percentage; Balb: *Baccharis albilanosa*; Bcor: *B. coridifolia*; Beri: *B. erigeroides*; Bnap: *B. napaea*; Boch: *B. ochracea*; Bplu: *B. pluricapitulata.*

methyl-5-hepten-2-one, 2-pentylfuran, myrcene + ethyl hexanoate, *p*-cymene, limonene, *trans*-2-octenal, *n*-octanol + *cis*-linalool oxide, *p*-cymenene, nonanal, linalool, *trans*-verbenol, myrtenal, α-terpineol, *cis*-carveol, cumin aldehyde, carvone, ethyl nonanoate, α-cubebene, *trans*-β-damascenone, cyclosativene, α-copaene, sativene, β-elemene, ylanga-2,4(15)-diene, α-gurjunene, *trans*-caryophyllene, β-copaene, neryl acetone, *allo*-aromadendrene, *trans*-β-ionone, γ-muurolene, germacrene D,

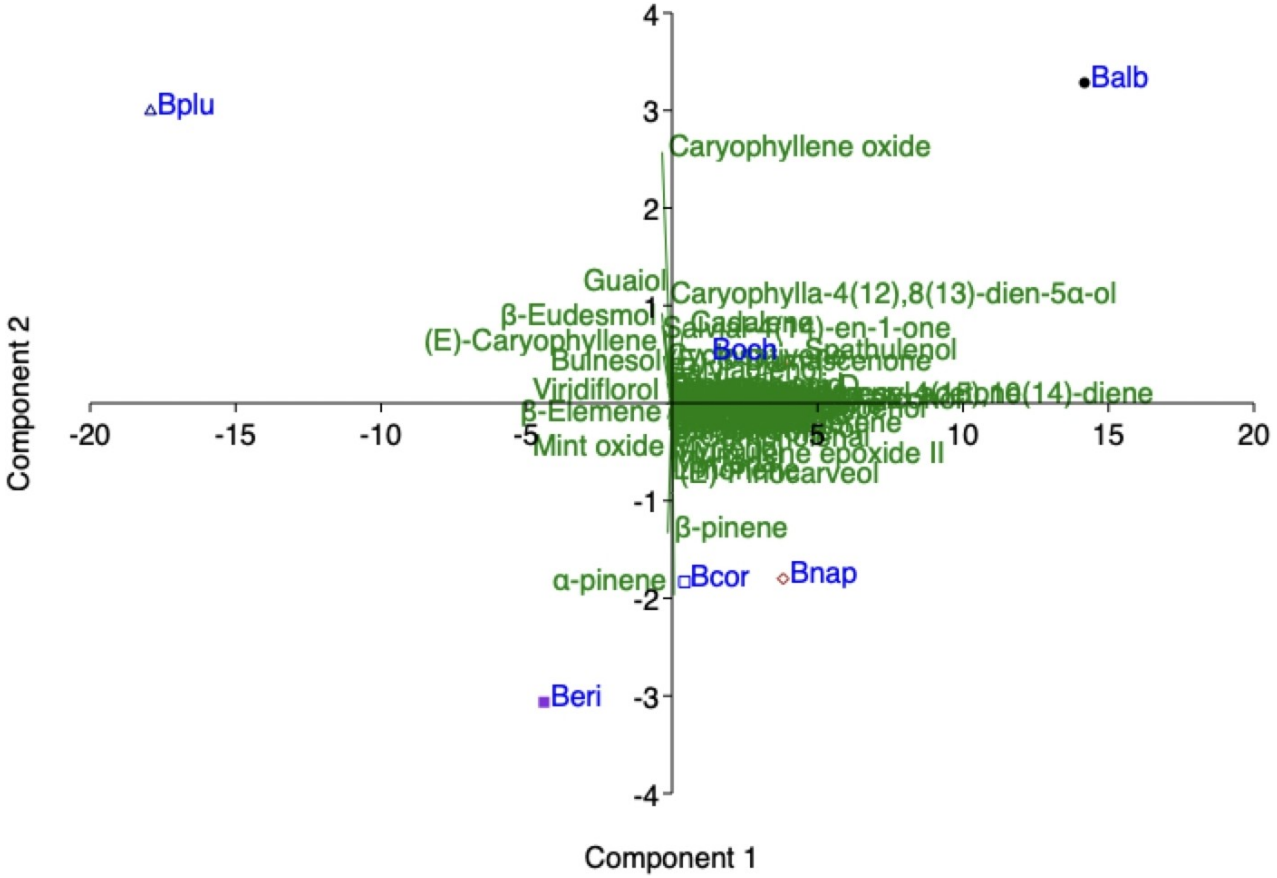

Figure 3. Biplot graph of principal component analysis (PCA1 = 87 % and PCA 2 = 7 %) based on six essential oil samples from *Baccharis* genus, and 172 chemical compounds (loading) identified from chromatographic analyses (GC/MS), for the species Balb = *B. albilanosa*, Bcor = *B. coridifolia*, Beri = *B. erigeroides*, Bnap = *B. napaea*, Boch = *B. ochracea* and Bplu = *B. pluricapitulata.*

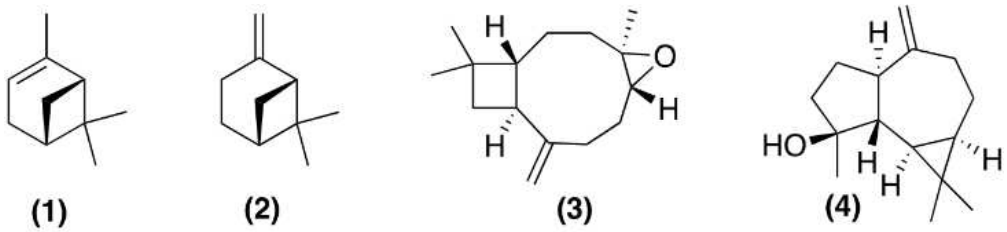

Figure 4. Structures of α-pinene (1), β-pinene (2), Caryophyllene oxide (3), Spathulenol (4).

β-selinene, α-muurolene, γ-cadinene, *trans*-calamenene, δ-cadinene, α-calacorene, salviadienol, β-calacorene, mint oxide, caryophyllenyl alcohol, spathulenol, caryophyllene oxide, globulol, salvial-4(14)-en-1-one, viridiflorol, humulene epoxide II, torilenol, 1,10-di-*epi*-cubenol, caryophylla-4(12),8(13)-dien-5α-ol, alismol, α-muurolol, 14-hydroxy-δ-cadinene, hexahydrofarnesyl acetone, neophytadiene, *trans*,*trans*-5,9-farnesyl acetone, hexadecanoic acid and ethyl hexadecanoate; were all present in the six studied species, with slight differences in abundances (Table 1). From the named components, the sesquiterpene salviadienol has not been previously reported from any *Baccharis* species to our knowledge. Thus it could be suggested as a possible chemomarker for the *Coridifoliae* subgenus. The corresponding ketone salvial-4(14)-en-1-one was previously reported at a trace level for *B. trinervis* Pers. EO from Costa Rica.[24]

Analyzing the species individually, the occurrence of the following components differentiate the species: *n*-decanol (0.03 %), bornyl acetate (0.09 %), undecanal (0.15 %) and agarospirol (0.28 %) in *B. albilanosa*; *trans*-pinocamphone (0.04 %), *cis*-chrysanthenyl acetate (0.06 %) and α-terpinen-7-al (0.01 %) in *B. coridifolia*; α-pinene oxide (0.06 %), neral (0.03 %), silphiperfol-5-ene (0.08 %), 7-*epi*-silphiperfol-5-ene (0.09 %), silphiperfola-4,7(14)-diene (0.08 %), α-amorphene (0.13 %), liguloxide (0.12 %), *trans*-nerolidol (0.38 %) and cubeban-11-ol (0.59 %) in *B. erigeroides*; benzene acetaldehyde (0.01 %), 1-undecene (0.26 %), 1-tridecene (0.09 %) and α-ylangene (0.12 %) in *B. napaea*; carvacrol (0.04 %), eugenol (0.06 %), tetradecanal

(0.16%), *epi*-α-muurolol (0.26%) and octadecanal (0.03%) in *B. ochracea*; and finally 1-hexen-6-ol (trace-level), n-heptanol (0.01%), *cis*-β-damascenone (0.02%), isoledene (0.09%), β-gurjunene (0.04%), 7-*epi*-α-eudesmol (0.50%), bulnesol (0.78%), β-bisabolol (0.27%), guaiol (2.48%) and ethyl *trans*-9-hexadecenoate (0.05%) in *B. pluricapitulata*. Since the EOs of each studied species show these distinctive constituents, they eventually could be chosen and suggested as chemical markers for these species (Table 1), supporting the species identification within *Coridifoliae*.

Baccharis albilanosa and *B. ochracea* were observed to be morphoanatomically and genetically closely related species by Almeida et al. (2021)[13] and Heiden et al. (2019),[25] respectively. In the present study, despite very similar chemical profiles, it was possible to differentiate the chemical compositions of these species (Table 1) in addition to the suggested chemical markers mentioned above. For example, *B. albilanosa* EO presented tricyclene (0.03%), hotrienol (0.01%), *p*-methylacetophenone (0.02%), octanoic acid (0.02%), 2,6-dimethyl-naphthalene (0.08%), *trans*-cadina-1(6),4-diene (0.05%), tridecanal (0.05%), cadalene (4.52%), pentadecanoic acid (0.08%), whereas these compounds were absent in *B. ochracea* EO (Table 1). On the other hand, *B. ochracea* EO exhibited β-pinene (0.47%), γ-terpinene (0.02%), terpinolene (0.03%), borneol (0.03%), *p*-cymen-8-ol + terpinen-4-ol (0.36%), *trans*-*p*-mentha-1(7),8-dien-2-ol (0.04%), verbenone (0.09%), geraniol (0.05%), perilla aldehyde (0.05%), geranial (0.04%), perilla alcohol (0.06%), modheph-2-ene (0.03%), β-bourbonene (0.03%), γ-gurjunene (0.11%), junenol (0.37%), β-eudesmol (0.64%) and pentadecanal (0.08%) which were not found in *B. albilanosa* (Table 1).

In comparison to previous studies, Bailac et al. (2001),[26] working with flowering female plant material of *B. coridifolia* from San Luis Province (Argentina), obtained an EO composition completely different from the present study, with isocaryophyllene (34.3%) and β-caryophyllene (10.8%) as the main components. Interestingly, a previous study performed with *B. coridifolia* plant collected at the full flowering stage in the Paraná State (Brazil) also showed also a different EO profile when compared to the present results since. In the aforementioned study, germacrene D and bicyclogermacrene (23.7 and 17.1%, respectively) were presented as the main components.[8] On the other hand, also for *B. coridifolia* material collected in Paraná State, Besten et al. (2012)[27] reported spathulenol and caryophyllene oxide (*ca.* 36 and 16%, respectively) as the major components of the EO obtained from the flowering aerial parts of male and female individuals, corroborating the findings of the present study. Altogether this data suggests the presence of more than one chemical race or chemotype for *B. coridifolia*, or a strong dependence of the oil composition with the environmental, seasonal, circadian, phenological, or physiological/genetic influences.[5,17] Nevertheless, it is necessary to investigate further to understand whether these variations in *B. coridifolia* are possibly linked to the named factors.

In the case of *B. ochracea* aerial parts' EO from the Rio Grande do Sul Brazilian State, Budel et al. (2012)[28] reported a composition with spathulenol and caryophyllene oxide (37.1 and 30.8%, respectively) as the main components, also supporting the results of the present investigation. Similarly, Minteguiaga et al. (2018)[20] reported a similar profile for a volatile extract (employed technique: simultaneous distillation-extraction, SDE) obtained from *B. ochracea* aerial parts collected in Uruguay. No previous studies are available in the literature on *B. albilanosa*, *B. erigeroides*, *B. napaea*, and *B. pluricapitulata* EOs or volatile extracts. Also, no phytochemical research on these species has been published to date. It is worth mentioning that the results of this research are particularly relevant to enhancing scientific knowledge on the chemistry of essential oils/volatile extracts from *Baccharis* subgenus *Coridifoliae*.

It is important to highlight that a homologous series of C_{10}-C_{18} fatty acids and some of its derivatives (methyl and ethyl esters, alcohols, ketones, and aldehydes) were identified in this work for all species under investigation (Table 1). These components are not usual constituents of EOs due to their low vapor pressure (semi-volatiles). These are natural metabolites that are shorter chain length compounds that are present in cuticular waxes of plants.[29,30] Since the extraction time employed in this work was 6 h in order to obtain an exhaustive extraction of the plant material, these components were able to vaporize, being dragged off by the water steam, and finally, condensate as EO components of the studied species. In nature, these wax components located at the interface plant-atmosphere protect against biotic (i.e., phytophagous) stress factors as well as abiotic stress factors, thus being considered as bioactive metabolites.[29,30]

Cytotoxic Potential of *Baccharis* subgenus *Coridifoliae* EOs

Screening natural products for potential cytotoxic effects is a strategy usually employed in the drug discovery process, especially when investigating new anticancer agents.[33,34] Therefore, an *in vitro* colorimetric assay against four cancer cell lines and two normal kidney cell lines was carried out in order to evaluate the cytotoxic potential of the EOs obtained from the six *Baccharis* species studied.

Our results demonstrate that the EOs of *B. albilanosa*, *B. coridifolia*, *B. napaea*, *B. ochracea* and *B. pluricapitulata* were cytotoxic against the four cancer cell lines with IC_{50} values ranging from 43–81 μg/mL for SK-MEL, 57–90 μg/mL for KB, 47–75 μg/mL for BT-549, and 39–70 μg/mL for SK-OV3 cells (Table 2). *Baccharis erigeroides* EO was the only one that did not show cytotoxicity against the four cancer cell lines at all concentrations tested (100–3.13 μg/mL). For the normal kidney cells, most samples were cytotoxic against LLC-PK1 and Vero cell lines with IC_{50} values ranging from 37–78 μg/mL and 60–70 μg/mL (Table 2), respectively, except for *B. erigeroides*, which was not cytotoxic against Vero cell lines. In another study, Montanha et al. (2004)[35] obtained similar cytotoxic data in which the aqueous and hydroethanolic extracts of *B. erigeroides* were not cytotoxic against normal Vero cells. As expected, doxorubicin was cytotoxic against all the cell lines at all the concentrations tested.

Table 2. Half-maximal inhibitory concentration (IC_{50}) of essential oils from the six *Baccharis* species studied against cancer (SK-MEL, KB, BT-549, and SK-OV-3) and normal cell lines (LLC-PK1 and Vero).

Samples	SK-MEL	KB	BT-549	SK-OV-3	LLC-PK1	Vero
B. albilanosa	70	80	63	61	43	70
B. coridifolia	52	62	47	39	37	60
B. erigeroides	>100	>100	>100	>100	78	>100
B. napaea	50	57	50	43	40	60
B. ochracea	43	90	51	47	73	70
B. pluricapitulata	81	71	75	70	42	70

Data expressed in IC_{50} μg/mL (half-maximal inhibitory concentration); SK-MEL: melanoma cell lines; KB: ubiquitous KERATIN-forming tumor cell lines; BT-549: human breast cancer cells; SK-OV-3: ovarian cancer cell lines; LLC-PK1: pig kidney epithelial cells; Vero: African green monkey kidney epithelial cells.

To the best of our knowledge, this is the first study evaluating the cytotoxic potential of *B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea*, and *B. pluricapitulata* EOs against BT-549, SK-MEL, SK-OV-3, and KB cancer cells and LLC-PK1 and Vero normal cells. Interestingly, *B. ochracea* presented promising results as the IC_{50} values for SK-MEL, SK-OV-3, and BT-549 cancer cells were significantly lower when compared to the IC_{50} values obtained for Vero and LLC-PK1 normal cells, suggesting a certain selectivity against cancer cells.

In addition, selectivity indices (SI) of the *Baccharis* EOs against the four cancer cell lines were calculated, and the results are summarized in Table 3. Some previous studies have reported that SI values above 2 indicate that the evaluated substance shows a suitable opportunity to become a new anticancer drug candidate.[36,37] Although *B. ochracea* showed interesting SI values against SK-MEL, BT-549, and SK-OV-3 in this study, values were lower than 2. Therefore, these results indicate the prerequisite of using a suitable drug delivery system for further biological use of these EOs to avoid the harmful effect on normal cells.

Previous studies have also demonstrated interesting results regarding the cytotoxic potential of other species, such as *B. ochracea* and *B. coridifolia* extracts, against different cancer cell lines. Monks et al. (2002)[11] revealed an outstanding cytotoxic potential of the aqueous and ethanolic extracts from the leaves and stems of *B. ochracea*, as the IC_{50} values against HT29 (human colon adenocarcinoma), U373 (human glioblastoma astrocytoma), and NCI-H460 (human large cell lung carcinoma) were below 5 μg/mL. Moreover, previous reports have shown potent cytotoxic effects of *B. coridifolia* dichloromethane and ethanol extracts against KB cells,[38] and ethanolic extract against acute lymphoblastic leukemia (CCRF-CEM) and chronic myelogenous leukemia cells (K562), with IC_{50} values ranging from 0.37 and 0.51 μg/mL, respectively.[39] Therefore, these findings corroborate the results obtained in this study, indicating cytotoxic potential in all *B. coridifolia* and *B. ochracea* extracts considered (volatile or not).

Despite the presence of sesquiterpenes, the mechanisms underlying their antineoplastic effects remain poorly understood. One can assume that β-caryophyllene acts by binding to cannabinoid receptor type 2. In contrast, β-caryophyllene oxide

Table 3. Selectivity Index of six *Baccharis* essential oils tested against cancer (SK-MEL, SK-OV-3, BT-549, and KB) and normal (LLC-PK1 and Vero) cell lines.

Samples	Cancer cell lines			
	SK-MEL	KB	BT-549	SK-OV-3
B. albilanosa	SI LLC-PK1 = 0.6	SI LLC-PK1 = 0.5	SI LLC-PK1 = 0.6	SI LLC-PK1 = 0.7
	SI Vero = 1.0	SI Vero = 0.8	SI Vero = 1.1	SI Vero = 1.1
B. coridifolia	SI LLC-PK1 = 0.7	SI LLC-PK1 = 0.5	SI LLC-PK1 = 0.7	SI LLC-PK1 = 0.9
	SI Vero = 1.1	SI Vero = 0.9	SI Vero = 1.2	SI Vero = 1.5
B. erigeroides	SI LLC-PK1 = NC	SI LLC-PK1 = NC	SI LLC-PK1 = NC	SI LLC-PK1 = NC
	SI Vero = NC	SI Vero = NC	SI Vero = NC	SI Vero = NC
B. napaea	SI LLC-PK1 = 0.8	SI LLC-PK1 = 0.7	SI LLC-PK1 = 0.8	SI LLC-PK1 = 0.9
	SI Vero = 1.2	SI Vero = 1.0	SI Vero = 1.2	SI Vero = 1.4
B. ochracea	SI LLC-PK1 = 1.7	SI LLC-PK1 = 0.8	SI LLC-PK1 = 1.4	SI LLC-PK1 = 1.5
	SI Vero = 1.6	SI Vero = 0.7	SI Vero = 1.3	SI Vero = 1.5
B. pluricapitulata	SI LLC-PK1 = 0.5	SI LLC-PK1 = 0.5	SI LLC-PK1 = 0.5	SI LLC-PK1 = 0.6
	SI Vero = 0.8	SI Vero = 0.9	SI Vero = 0.9	SI Vero = 1.0

BT-549: human breast cancer cells; KB: ubiquitous KERATIN-forming tumor cell lines; LLC-PK1: pig kidney epithelial cells; NC: not calculable; SI: selectivity index; SK-MEL: melanoma cell lines; SK-OV-3: ovarian cancer cell lines; Vero: African green monkey kidney epithelial cells.

does not exhibit affinity to cannabinoid receptor type 1/2, but reveals equally strong or even stronger anticancer activity than β-caryophyllene. It is known that β-caryophyllene oxide modifies numerous key pathways for cancer development, such as mitogen-activated protein kinase, phosphoinositide 3-kinase (MAPK), PI3 K/AKT/mTOR/S6 K1, and Signal Transducer and Activator of Transcription 3 (STAT3) pathways. Furthermore, treatment with this compound decreases the expression of pro-cancer genes/proteins, while increasing levels of those with proapoptotic properties.[40]

In order to exert its cytotoxic action, the sesquiterpene spathulenol relies on apoptosis.[41] These results shown in the present research can be due to the synergistic interaction among monoterpenes and sesquiterpenes present in the EOs. Accordingly, the activity of the main compounds can be affected by other small molecules, synergism appears to be more substantial than its individual compounds owing to the possibility of improving cellular dispersion of the EOs.[42]

Conclusions

To the best of our knowledge, this is the first report to analyze and compare the chemical compositions of EOs from *B. albilanosa*, *B. erigeroides*, *B. napaea*, and *B. pluricapitulata*. Conforming to our results, the EOs of all the six species studied presented spathulenol and caryophyllene oxide as the main components, indicating that there is a qualitative similarity between the volatile expression of these species because they belong to the same subgenus. Interestingly, some components were only observed in one species, emphasizing that such compounds could be useful as chemical markers for differentiating these six species of *Baccharis*.

It is worth mentioning that *B. albilanosa* and *B. ochracea* are closely allied species with similar chemical profiles. However, it was possible to notice some differences, in addition to the chemical markers, that differentiate these two species chemically.

Finally, our findings suggest that *B. ochracea* essential oil offers more promising results, as the IC_{50} values for cancer cells were lower than the IC_{50} values for the normal cell lines.

Experimental Section

Botanical material

Fresh specimens of vegetative aerial parts were collected in triplicate from *Baccharis albilanosa* A.S.Oliveira & Deble, *B. coridifolia* DC., *B. erigeroides* DC., *B. napaea* G.Heiden, *B. ochracea* Spreng., and *B. pluricapitulata* (Deble) G.Heiden in April 2018 and 2019 from sunny and open areas in grassland ecosystems from Rio Grande do Sul, Brazil. The herbarium specimens were registered, identified, and deposited in the Herbarium of Embrapa Clima Temperado (ECT) (Rio Grande do Sul, Brazil) by the taxonomist Dr. Gustavo Heiden. The collection sites georeferencing and the herbarium specimen numbers are provided in Table 4. The access to the botanical source was authorized and licensed by the *Conselho de Gestão do Patrimônio Genético* (CGEN/SISGEN) registered under code AB29E78. The samples collected underwent stabilization and drying in an oven at 30 °C before analysis.

Extraction of essential oils (EOs)

Essential oils (EOs) were extracted from 100 g of dried plant material (leaves and stems) by hydrodistillation for 6 h (water-to-plant material ratio of 2 L:100 g), in triplicate, using a Clevenger-type apparatus. The EOs obtained were dried on anhydrous Na_2SO_4, stored in amber glass vials with Teflon-sealed caps, and refrigerated at −4 °C for further analysis. The EOs yield was calculated as volume/mass percentage.[43]

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The EOs of *B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea*, and *B. pluricapitulata* were analyzed by GC/MS using an Agilent 7890 A GC system equipped with a 5975 C quadrupole mass spectrometer and a 7693 autosampler (Agilent Technologies, Santa Clara, CA, USA). Dilutions (1:100) in hexane of EOs were performed for each oil sample, and 1 μL of the solution was injected. Separation was performed on a DB-5MS (Agilent J&W Scientific, Folsom, CA, USA) capillary column (30 m×0.25 mm i.d. ×0.25 μm film thickness). The oven temperatures were programmed as follows: 45 °C (2 min), 45–130 °C at 2 °C/min, 130 °C (10 min), 130–280 °C at 2 °C/min, 280 °C (10 min). The injector temperature was 280 °C; the carrier gas was helium (1 mL/min of flow rate); the injection mode was split with a 1:50 ratio. Mass spectra were recorded at 70 eV in scan mode from *m/z* 35 to 500. The transfer line, ion source, and quadrupole temperatures were set at 280 °C, 230 °C, and 150 °C, respectively. Data collection was performed using the Agilent MSD Chemstation (F.01.03.2357). Each analysis was performed in triplicate.

The identification of the individual compounds was based on the comparison of their GC linear retention index (LRI) in the named non-polar column and on the mass spectra match of the

Table 4. Location, coordinates and herbarium registry of samples of the six studied species of *Baccharis*.

Species	Location and Coordinates	Herbarium number
B. albilanosa	Brazil, Rio Grande do Sul, Manoel Viana. 29°59′33″S, 55°37′63″W, 127 m.	ECT777979
B. coridifolia	Brazil, Rio Grande do Sul, São José dos Ausentes. 28°52′33″S, 49°75′97″W, 1196 m.	ECT005992
B. erigeroides	Brazil, Rio Grande do Sul, São José dos Ausentes. 28°52′33″S, 49°75′97″W, 1196 m.	ECT005997
B. napaea	Brazil, Rio Grande do Sul, São José dos Ausentes. 28°52′33″S, 49°75′97″W, 1196 m.	ECT005991
B. ochracea	Brazil, Rio Grande do Sul, Pedras Altas. 31°81′49″S, 53°55′49″W, 313 m.	ECT780495
B. pluricapitulata	Brazil, Rio Grande do Sul, Pedras Altas. 31°81′49″S, 53°55′49″W, 313 m.	ECT783953

components with dedicated libraries[31,32,44–46] and with previously published data from our research group.[14,20–22,28] The abundances of each component were obtained as the raw percentage peak area of each compound from the full scan GC/MS chromatograms. Additional standardization was not performed since the focus of this research was to identify EO volatile compounds for the differentiation of the species.

Statistical analysis

Principal component analysis and hierarchical grouping were performed using the PAST program, version 3.13.12. The data used for the multivariate analyses were the dependent variables [compounds of the essential oils and class such as monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OS) and diterpene (DIT)], while the independent variables were essential oil samples based on species.

Cytotoxicity assay

To evaluate the cytotoxic potential of *B. albilanosa*, *B. coridifolia*, *B. erigeroides*, *B. napaea*, *B. ochracea*, and *B. pluricapitulata* essential oils, four human cancer cell lines (SK-MEL melanoma cell lines, KB ubiquitous KERATIN-forming tumor cell lines, SK-OV-3 ovarian cancer cell lines, and BT-549 human breast cancer cells) and two normal kidney cell lines (LLC-PK1 pig kidney epithelial cells and Vero African green monkey kidney epithelial cells) were used.

All cell lines were grown in fresh RPMI® 1640 medium (pH 7.2) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic. Briefly, cells were seeded in 96-well plates at a density of 10.000 cells/well followed by incubation to allow cell attachment at 37 °C provided with 5% CO_2 for 24 h to allow stabilization, production of extracellular matrix, and adhesion of cells to the plate. In parallel, a stock solution was prepared by dissolving essential oil samples in dimethyl sulfoxide (DMSO) at 20 mg/mL.

Each stock solution was diluted in the medium, and six final concentrations were reached (100, 50, 25, 12.5, 6.25, and 3.13 μg/mL). The same procedure was performed for DMSO (negative control) and doxorubicin (positive control). After this procedure, plates were carefully placed in the incubator for 48 h, then removed, and 10 μL of cell counting kit-8 (CCK-8) solution was added to each well. Plates were incubated for an additional period of 45 min to 1 h, and absorbances at 450 nm were recorded using a microplate reader. The percentage viability of cells treated with the different concentrations of each sample was calculated in comparison to DMSO control cells. The half-maximal inhibitory concentration (IC_{50}) was calculated from the concentration-response curves.

Results were statistically evaluated by analysis of variance (one-way ANOVA) followed by post hoc Dunnett's test. Significance was considered when $p < 0.05$. In addition, the IC_{50} values were applied to perform the Selectivity Index (SI) determination, in which the SI was calculated as the ratio between the IC_{50} values calculated for normal cells divided by the IC_{50} calculated for tumoral cells.

Abbreviations

Balb – *Baccharis albilanosa*. Bcor – *Baccharis coridifolia*. Beri – *Baccharis erigeroides*. Bnap – *Baccharis napaea*. Boch – *Baccharis ochracea*. Bplu – *Baccharis pluricapitulata*. BT-549 – Human breast cancer cells. CCRF-CEM – Acute lymphoblastic leukemia. CGEN/SISGEN – Conselho de Gestão do Patrimônio Genético. DIT – Diterpene. DMSO – Dimethyl sulfoxide. ECT – Embrapa Clima Temperado. EO – Essential oil. EOs – Essential oils. FBS – Fetal bovine serum. GC/MS – Gas Chromatography-Mass Spectrometry. HT29 – Human colon adenocarcinoma. IC_{50} - Half-maximal inhibitory concentration. K562 – Chronic myelogenous leukemia cells. KB – Ubiquitous KERATIN-forming tumor cell lines. LLC-PK1 – Pig kidney epithelial cells. MH – Monoterpene hydrocarbon. NCI–H460 – Human large cell lung carcinoma. OM – Oxygenated monoterpene. OS – Oxygenated sesquiterpenes. SDE – Simultaneous distillation-extraction. SH – Sesquiterpene hydrocarbon. SI – Selectivity indices. SK-MEL – Melanoma cell lines. SK-OV-3 – Ovarian cancer cell lines. U373 – Human glioblastoma astrocytoma. Vero – African green monkey kidney epithelial cells.

Author Contributions

Collection and identification of plant materials, G.H.; Extraction of EOs, V.P.A.; GC/MS, M.W., J.M.; Chemical profile, V.P.A. M.W. M.M., E.D. and J.M.; Biplot graph analysis, D.S.A.C.; Cytotoxic activity, S.E.L.T., S.I.K., J.T.; Critical reading and insightful recommendations of the manuscript, V.R., P.V.F and I.A.K. Supervision of the laboratory work, J.M., A.G.J. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

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