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Chemical, Antioxidant, and Antimicrobial Evaluation of Essential Oils and an Anatomical Study of the Aerial Parts from *Baccharis* Species (Asteraceae)

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The aim of this study was to evaluate the chemical, antioxidant, and antimicrobial activity of the essential oils as well as the anatomy of the aerial parts from *Baccharis aracatubaensis*, *Baccharis burchellii*, and *Baccharis organensis* owing to the therapeutic potential of *Baccharis*. The volatile constituents were analyzed using GC/MS, the antioxidant activity was evaluated by oxygen radical absorbance capacity ($ORAC_{FL}$) and DPPH assays, and the antimicrobial activity by a microdilution technique. Of the 56 compounds identified, only seven (β-caryophyllene, γ-muurolene, bicyclogermacrene, β-germacrene, spathulenol, τ-muurolol, and α-cadinol) were common in the three specimens studied. Of these, γ-muurolene was found abundantly in *B. aracatubaensis*, while bicyclogermacrene was abundant in *B. burchellii* and *B. organensis*. The essential oils exhibited antioxidant activity in the $ORAC_{FL}$ ($> 500.0\ \mu mol\,TE\,g^{-1}$) and DPPH assays. However, they did not exhibit any antimicrobial activity. Secretory ducts and flagelliform glandular trichomes were observed in the anatomical study of all the *Baccharis* species studied.

Keywords: *Baccharis*, essential oils, terpenoids, anatomical study, antimicrobial activity, antioxidant activity, biological activity.

Introduction

Asteraceae is among the largest species-rich families within the angiosperms, comprising of approximately 23,000 species belonging to 1,600 genera.[1] Between these genera stands out *Baccharis* L., which is one of the 10 most diverse genera of the family, with approximately 400 species distributed from Canada to Southern Argentina and Chile.[2] In Brazil, there are 178 species currently present across all biomes and are found to be more abundant in the South, Southeast and Midwest regions.[3] Among these, *Baccharis aracatubaensis* Malag. is included in the vulnerable and almost endangered Red list under the classification,[3] justifying the importance of studies with this species. *B. aracatubaensis* popularly known as broom is a subshrub native to Brazil that belongs to the *Axillaris* section, growing approximately up to 1.2 m high, and restricted to the states of Santa Catarina and Paraná.[4] *Baccharis burchellii* Baker, popularly known as 'alecrim-carqueja' belongs to the *Caulopterae* section. It is exclusively distributed in Brazil, being endemic to the Southeastern Region (Minas Gerais, Rio de Janeiro, and São Paulo), and occurring in the Atlantic Forest Biome.[5] *Baccharis organensis* Baker, which belongs to the *Caulopterae* section, is popularly known as broom,

and is a branched shrub native to Brazil that grows approximately 1.0 h high, and is geographically distributed in Rio de Janeiro ('Serra dos órgãos' and 'Itatiaia'), Paraná, Santa Catarina, and Rio Grande do Sul.[4,5]

Asteraceae is considered to be one among approximately 50 families of plants that produce essential oils. In this family, oils are found in the anatomical structures. In *Baccharis*, secretory ducts are generally observed. Due to the great difficulty that arises while identifying plants from *Baccharis* species, several studies have been performed using these secretory structures to distinguish these species, especially the 'carquejas'. Many studies have examined its morpho-anatomical characteristics to determine the pharmacobotanical characters of the taxon and support the quality control of vegetable drugs.[6–9]

According to a review realized in 2016 by *Campos* and collaborators,[10] the plants of the genus *Baccharis* are rich in volatile organic compounds. However, approximately 50 species had the chemical composition of the essential oils studied. There have been few studies on chemical characterization, according to male and female specimens separately.[11–13]

Several pharmacological activities are attributed to essential oils, including antioxidant, antimicrobial, anti-inflammatory, antifungal, acetylcholinesterase, antiprotozoal, analgesic, antiparasitic, sedative, insecticidal, antitrypanosomal, and antitumor.[10,14–19] In addition, these oils are widely used as fragrance in cosmetics and cleaning products, as well as use in food and medicine as additives, because of their antioxidant, antimicrobial, and flavoring properties.[20–29]

Among *Baccharis* species, the most studied species of high socio-economic value is *Baccharis dracunculifolia* DC., which is known to be the main plant source of Brazilian green propolis. It is widely used for its antimicrobial, anti-inflammatory, and analgesic effects.[30,31] This species also produces (*E*)-nerolidol, a high value compound for the perfume industry.[32] *Baccharis salicifolia* (Ruiz & Pav.) Pers. and *Baccharis pilularis* DC. produce a nectar that leads to the production of high quality honey.[33] *Baccharis trimera* (Less.) DC. is widely used in folk medicine as a digestive aid, diuretic, hepatoprotective, anti-inflammatory, and antihypertensive agent, as well as in detoxification and control of obesity,[34–36] and also produce carquejil acetate of high value for the perfume industry.[32]

Due to the ethnobotanical and pharmacological importance of *Baccharis* and the concomitant depletion of species in the Brazilian biomes, study of *Baccharis* species holds great importance. Therefore, the aim of this study was to evaluate the chemical, antioxidant, and antimicrobial activity of essential oils obtained from the flowers of male and female specimens and the study of the anatomy of secretory structures of the aerial vegetative organs of *B. aracatubaensis*, *B. burchellii*, and *B. organensis*.

Results and Discussion

The inflorescence of the male and female specimens of *B. aracatubaensis*, *B. burchellii*, and *B. organensis* yielded a light yellow crude essential oil. The yield in relation to the dry weight of the plant material for male and female specimens were 0.17% and 0.14% for *B. aracatubaensis*, 0.10% for both the specimens of *B. burchellii* and *B. organensis*, respectively.

As shown in *Table 1*, 56 compounds were identified in the chemical composition of the essential oils from the three *Baccharis* species studied. The essential oils of these species revealed a high proportion of sesquiterpenes in male and female specimens of *B. aracatubaensis* (89.2% and 100%, respectively), *B. burchellii* (100% for both the specimens), and *B. organensis* (99.0% and 91.2%, respectively). The monoterpenes were observed in small amounts in all the analyzed specimens, while α-thujene, sabinene, and limonene were observed only in the male specimens of *B. aracatubaensis* (10.3%) and in the female specimens of *B. organensis* (6.8%). However, in the male specimens of *B. organensis*, only α-thujene and sabinene were observed (0.9%). Monoterpenes were not found in *B. burchellii*. Of the 56 compounds identified, only seven compounds ((β)-caryophyllene, γ-muurolene, bicyclogermacrene, β-germacrene, spathulenol, τ-muurolol, and α-cadinol) were common in all the specimens analyzed.

According to the results obtained by HCA analysis (*Figure 1*), similarities were observed among the male and female specimens of the *Baccharis* species studied. There are only a few studies comparing male and female specimens despite a large population of dioecious species in *Baccharis*. These studies show that there may or may not be differences in the chemical composition of the essential oils of male and female *Baccharis* specimens.[11–13,39–42] However, with HCA analysis (*Figure 1*), it was possible to observe a great similarity in the composition between the essential oils from *B. aracatubaensis* and *B. organensis*, which are the representatives of different sections, *Axillaris* and *Caulopterae*, respectively. This similarity can be explained by the stage of plant development and

Table 1. Essential oil composition of male and female specimens from *Baccharis aracatubaensis*, *B. burchellii*, and *B. organensis*.

Compounds		RI[a]	RI[b]	*B. aracatubaensis*		*B. burchellii*		*B. organensis*	
				Male	Female	Male	Female	Male	Female
1	α-Thujene	930	929	2.3	–	–	–	0.6	0.3
2	Sabinene	970	971	2.5	–	–	–	0.3	2.9
3	**Limonene**	1024	1024	**5.5**	–	–	–	–	3.6
4	α-Terpinolene	1088	1085	–	–	–	–	–	0.5
5	α-Terpineol	1189	1189	–	–	–	–	–	1.0
6	Silphiperfol-5-ene	1328	1325	–	–	–	0.7	–	–
7	α-Longipinene	1352	1361	–	–	–	0.2		
8	Cyclosativene	1371	1370	1.3	1.6	–	–	0.2	0.2
9	α-Ylangene	1375	1370	–	–	0.2	2.3	–	–
10	β-Panasinsene	1382	1387	–	–	2.4	1.5	–	–
11	β-Cubebene	1387	1384	0.4	0.4	–	–	–	–
12	β-Elemene	1390	1388	0.4	0.7	–	–	0.6	0.6
13	Sibirene	1400	1403	0.4	0.4	–	–	–	–
14	**β-Caryophyllene**	1418	1414	**10.9**	**12.7**	**6.9**	**11.8**	1.1	2.0
15	α-*trans*-Bergamotene	1431	1430	–	–	–	–	0.2	0.3
16	β-Copaene	1432	1429	–	–	0.1	0.2	–	–
17	Aromadendrene	1439	1434	–	–	0.6	0.6	1.2	1.2
18	α-Caryophyllene	1452	1449	0.8	0.9	–	4.6	0.2	2.5
19	*allo*-Aromadendrene	1460	1456	–	0.2	–	–	0.4	0.2
20	**γ-Muurolene**	1477	1477	**21.4**	**23.9**	**7.3**	**10.8**	**6.8**	**10.4**
21	γ-Himachalene	1482	1482	–	–	0.3	0.4	–	–
22	α-Amorphene	1484	1482	–	–	–	–	0.3	–
23	Germacrene D	1485	1487	0.2	–	–	–	–	–
24	**Bicyclogermacrene**	**1494**	**1493**	**21.4**	**21.7**	**20.7**	**17.8**	**15.2**	**15.1**
25	Viridiflorene	1496	1497	–	–	0.8	1.2	–	–
26	α-Muurolene	1500	1504	0.9	1.3	–	0.6	0.4	0.7
27	β-Bisabolene	1505	1501	0.6	0.6	1.2	0.8	–	–
28	**Selinene**	1522	1521	–	–	**5.6**	–	–	–
29	**δ-Cadinene**	1523	1521	**6.6**	**8.2**	–	–	2.1	3.2
30	(*E*)-Bisabolene	1529	1529	–	–	0.1	0.2	–	–
31	α-Calacorene	1545	1542	–	–	–	–	0.3	0.4
32	Hedycaryol	1546	1548	1.0	–	–	–	–	–
33	Elemol	1549	1548	–	–	2.3	0.8	–	–
34	β-Germacrene	1556	1554	0.7	0.7	0.2	1.5	1.4	3.4
35	Globulol	1570	1565	–	–	–	–	1.6	1.6
36	Spathulenol	1575	1576	2.9	3.9	4.3	2.0	4.7	4.5
37	Epiglobulol	1578	1582	–	–			7.4	6.4
38	**Thujopsan-2α-ol**	1587	1581	–	–	**9.1**	**6.4**	–	–
39	**Viridiflorol**	1590	1590	–	–	2.9	1.3	**23.0**	**11.9**
40	Ledol	1602	1602	1.3	1.9	–	–	3.9	4.8
41	Eudesmol	1607	1600	–	–	1.7	1.4	–	–
42	Humulene epoxide	1608	1607	–	–	0.3	0.8	–	–
43	1-*epi*-Cubenol	1628	1628	1.0	1.2	–	–	2.0	2.7
44	Eremoligenol	1631	1627	–	–	1.7	0.7	–	–
45	*cis*-α-Bisabolene epoxide	1640	1638	–	–	–	–	1.6	1.1
46	τ-Muurolol	1642	1642	3.2	3.8	7.9	0.2	1.2	2.2
47	Cubenol	1646	1641	–	–	0.6	8.2	–	–
48	α-Himachalene	1649	1652	–	–	–	–	–	0.2
49	β-Eudesmol	1650	1649	–	–	12.3	0.4	–	–
50	**α-Cadinol**	1654	1654	3.8	4.2	0.6	**9.3**	**7.5**	1.8
51	Shyobunol	1689	1690	2.1	1.1	–	–	–	–
52	Farnesol	1698	1690	–	–	1.6	0.3	–	–
53	Eudesm-7(11)-en-4-ol	1700	1696	–	–	–	–	1.1	0.9
54	**Curcuphenol**	1718	1723	–	–	–	–	**5.9**	4.8
55	**Mint sulfide**	1741	1737	3.2	**6.9**	–	–	–	–
56	Bisabolene	1749	1749	–	–	–	–	2.2	2.2

Table 1. (cont.)

Compounds	RI[a]	RI[b]	*B. aracatubaensis*		*B. burchellii*		*B. organensis*	
			Male	Female	Male	Female	Male	Female
Monoterpenoid hydrocarbons			10.3	–	–	–	0.9	7.3
Oxygenated monoterpenoids			–	–	–	–	–	1.0
Sesquiterpenoid hydrocarbons			66.0	73.3	46.4	55.2	30.3	40.4
Oxygenated sesquiterpenoids			15.3	16.1	45.3	31.8	62.1	44.9
Sulfur-containing sesquiterpenes			3.2	6.9	–	–	–	–
Total identified compounds [%]			94.8	96.3	91.7	87.0	93.3	93.5

[a] RI (calc.), retention indices on DB-5MS column calculated according to ref. [37].
[b] RI, retention indices according to ref. [38].

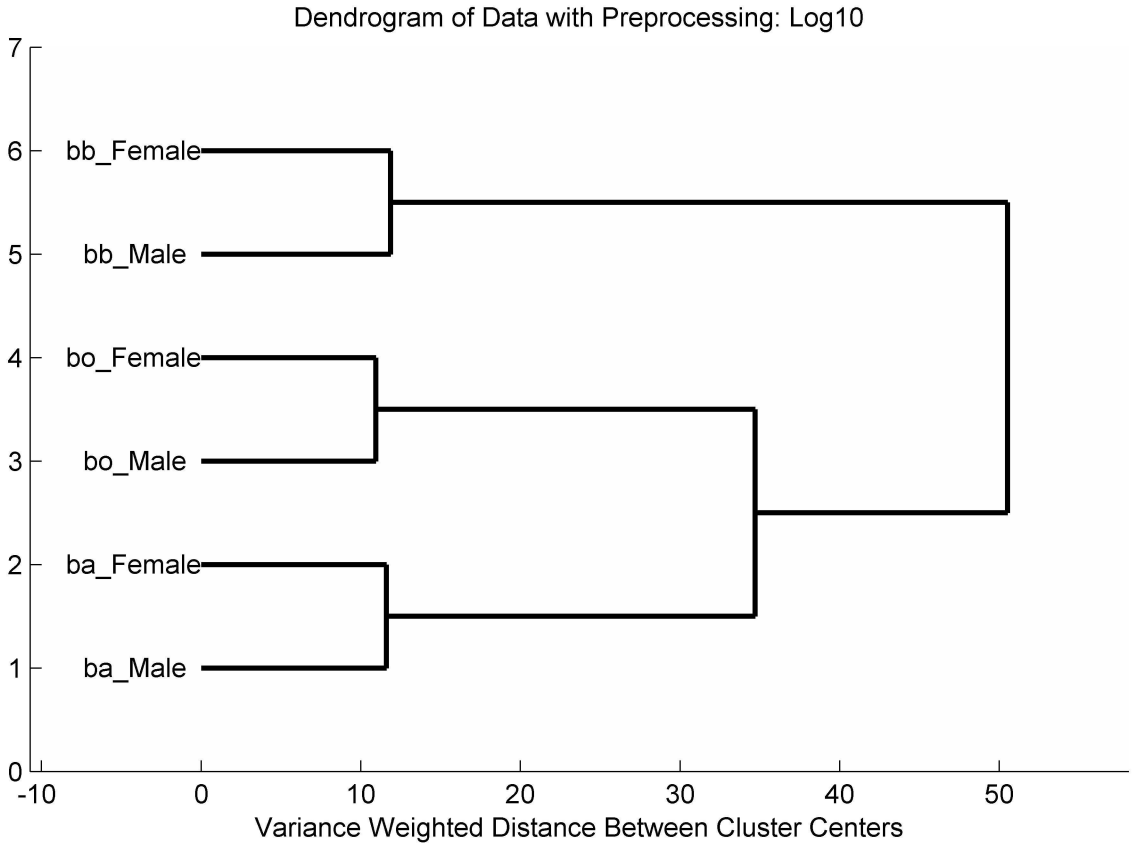

Figure 1. Hierarchical cluster analysis applied to *Baccharis aracatubaensis* (ba), *B. burchellii* (bb), and *B. organensis* (bo) essential oil samples (male and female specimens) using all identified compounds.

environmental conditions. *B. aracatubaensis* and *B. organensis* were collected in mountainous regions above 1000 m of altitude and under high solar irradiation, while *B. burchellii* was collected from a region with higher humidity (river proximity), lower luminosity, and altitude. In general, the biosynthesis of certain vegetable constituents, especially terpenoids, is genetically determined, but the influence of abiotic factors, such as light, temperature, water, soil, and altitude, has been proven in different species.[43,44]

Among the major compounds identified in the essential oils of the analyzed samples, many have been previously reported in other species of the genus *Baccharis*.[10] The compounds limonene, β-caryophyllene, α-cadinol, bicyclogermacrene, spathulenol, and viridiflorol have been identified in *Baccharis uncinella* DC.,[41,45,46] epiglobulol and β-eudesmol in *B.*

trimera;[47] curcuphenol in *Baccharis genistelloides* (LAM). PERS.;[48] γ-muurolene and δ-cadinene in *Baccharis dracunculifolia* DC.;[49] and limonene, β-caryophyllene, γ-muurolene, bicyclogermacrene, and spathulenol in *Baccharis semiserrata* DC.[46]

A recent study on the chemical composition of essential oils from five *Baccharis* species showed the presence of α-pinene in *Baccharis reticularioides* DEBLE and A.S. OLIVEIRA; α-bisabolol in *Baccharis punctulata* DC.; spathulenol and kongol in *Baccharis microdonta* DC., β-pinene and limonene in *Baccharis pauciflosculosa* DC., and β-pinene, limonene, and spathulenol in *Baccharis sphenophylla* DUSÉN ex MALM[19] as the major compounds. These essential oils were extracted from vegetative aerial parts of *Baccharis*.

The essential oils of these specimens of *Baccharis* possessed antioxidant/free-radical scavenging effects. The ORAC and DPPH results for the essential oils are summarized in *Table 2*.

Table 2. Antioxidant assay of the essential oils of male and female specimens from *Baccharis aracatubaensis*, *B. burchellii*, and *B. organensis*.

Samples (essential oil/experimental control)	ORAC assay[a]	DPPH assay[b]
B. aracatubaensis (Male)	680.6 (1.79)ε	Strong
B. aracatubaensis (Female)	536.1 (5.87)ε	Medium
B. burchellii (Male)	576.7 (2.38)ε	Medium
B. burchellii (Female)	626.0 (1.88)ε	Medium
B. organensis (Male)	1202.2 (4.77)ω	Strong
B. organensis (Female)	951.4 (1.04)ω	Strong
β-Caryophyllene[c]	1.4 (0.60)^{I}	Strong
Bicyclogermacrene[c]	1.2 (0.50)^{I}	Strong
Limonene[c]	2.0 (1.10)^{II}	Strong
α-Terpineol[c]	2.6 (1.20)^{II}	Strong
Quercetin[c]	5.5 (1.50)^{III}	Strong

[a] ORAC data expressed as μmol of Trolox equivalents per g of essential oil (μmol of TE g^{-1}), mean (% RSD, relative standard deviation) of triplicate assays. [b] TLC-based 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger antioxidant assay. [c] Positive experimental controls (Sigma-Aldrich) with ORAC data expressed as relative Trolox equivalent, mean (% RSD, relative standard deviation) of triplicate assays. Statistics: ($\varepsilon \neq \omega$) and ($I \neq II \neq III$), $P < 0.001$ (ANOVA and Tukey's post-hoc test).

In TLC autographic assay for DPPH radical scavenging (qualitative method), all samples produced yellow spots after the DPPH reagent was sprayed, suggesting antioxidant activity for these essential oils at 1:250 (v/v) dilution. In $ORAC_{FL}$ assay (quantitative method), the criteria used for interpretation of results of antioxidant capacity was: mixtures of compounds such as extracts and essential oils possess high antioxidant capacity with results in $ORAC_{FL}$ assay $\geq$ 800.0 μmol of Trolox equivalent (TE) g^{-1} and isolated compounds with values of $\geq$ 1.0 relative Trolox equivalent (RTE).[50–52] All samples tested showed antioxidant capacity greater than 500.0 μmol TE g^{-1}. In these essential oils were found β-caryophyllene, bicyclogermacrene, limonene, and α-terpineol compounds, and according to the literature, these are known for their potent antioxidant activity and commonly found in the composition of essential oils.[53–56]

Few studies have reported the antioxidant and antimicrobial activities of the essential oils of *Baccharis*.[10] Of the three species analyzed in this study, only *B. burchellii* has been reported to have antioxidant activity, however, the study was performed from the crude extract of the aerial parts.[57] The oil of *B. uncinella* showed inhibition in the formation of reactive oxygen species when assessed by the coupled oxidation of β-carotene and linoleic acid.[26] *B. dracunculifolia* and *Baccharis trinervis* PERS. showed inhibition in the formation of reactive oxygen species when assessed by the coupled oxidation of β-carotene and linoleic acid and DPPH assays.[18,26,58] The essential oil from *Baccharis tridentata* VAHL showed no significant antioxidant activity against the coupled oxidation of β-carotene and linoleic acid and DPPH assays.[59]

The antioxidant properties of essential oils from several plants have been evaluated using $ORAC_{FL}$ and DPPH assays in previous studies, and in these oils, γ-muurolene, δ-cadinene, germacrene D, bicyclogermacrene, α-copaene, and (β)-caryophyllene were found to be the major components.[51] In addition, significant *in vitro* antioxidant activities in the $ORAC_{FL}$ and DPPH assays were documented for the essential oils extracted from the leaves of *Annona salzmannii* A.DC. and *Annona pickelii* (DIELS) H.RAINER (Annonaceae), and sesquiterpenes predominated in both essential oils, with bicyclogermacrene, β-caryophyllene, α-copaene, germacrene D, and δ-cadinene[50] as main components. Therefore, the antioxidant capacity observed in the present study is also attributed to the sesquiterpenes from the essential oils of these *Baccharis* species. The highest antioxidant activity was observed for the essential oils from *B. organensis* (900–1203 μmol of TE g^{-1}), and this demonstrates the major chemistry diversity, as about 33 compounds were identified in the essential oils from this species, being the sesquiterpenes as predominant class. This activity has been attributed to the complex essential oils and rarely to isolated compounds.[24] The compounds curcuphenol, α-terpineol, epiglobulol, bisabolene, eu-

Table 3. Antimicrobial activity of the essential oils of male and female specimens from *Baccharis aracatubaensis*, *B. burchellii*, and *B. organensis*.

Microorganism	*B. aracatubaensis*[a]	*B. burchellii*[a]	*B. organensis*[a]	Controls[b]
S. aureus (ATCC 6538)	>1000	>1000	>1000	100
E. coli (ATCC 8738)	>1000	>1000	>1000	100
P. aeruginosa (ATCC 9027)	>1000	>1000	>1000	100
C. albicans (ATCC 10231)	>1000	>1000	>1000	500

[a] Concentrations are expressed in $\mu g\,mL^{-1}$. [b] Positive controls: chloramphenicol for bacterial strains and ketoconazole for yeast strains.

desm-7(11)-en-4-ol, *cis*-α-bisabolene epoxide, and globulol were identified only in *B. organensis*, suggesting that a possible synergism might be involved among the main components or with other minor components, as the essential oils are a complex mixture of components.[60]

To the best of our knowledge, this is the first report on the analysis of the constituents of the essential oils of *B. aracatubaensis*, *B. burchellii*, and *B. organensis*, and their antioxidant activity. The chemical compositions of these essential oils are in agreement with the chemistry of the oils reported previously in another species of *Baccharis*. The strong ($\geq 800.0\ \mu mol\,TE\,g^{-1}$) antioxidant activity of the essential oils obtained from *Baccharis* specimens studied suggests their potential as a natural source of biologically active compounds.

Many of these compounds show promising biological activities;[10] for instance, bicyclogermacrene, cubenol, and epiglobulol show antibacterial activity,[61–63] while viridiflorol and curcuphenol show antifungal activity.[64,65] Thus, the investigation of the antioxidant activity of the essential oils obtained from *Baccharis* species analyzed is crucial for the development of new pharmacologic agents for the treatment of diseases.

Antioxidant activity is related to a series of other activities[66–68] and the positive antioxidant results observed in the present study motivated us to perform the antimicrobial assay. However, no antimicrobial activity was observed against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* strains, as shown in *Table 3*.

Some species of *Baccharis* such as *B. dracunculifolia*[18,69] and *B. uncinella*[46] did not exhibit any antimicrobial activity. *B. semiserrata* showed weak antimicrobial activity for *S. aureus*.[46] The negative results obtained for *B. aracatubaensis*, *B. burchellii*, and *B. organensis* may be associated with little or no presence of monoterpenes in the essential oils. According to the results obtained by *Miranda et al.* (2016), the essential oils with higher content of monoterpenes were more effective at inhibiting bacterial growth than essential oils rich in sesquiterpenes.[18] The antibacterial activity presented by oils rich in monoterpenes can be justified by the ability of these compounds to reach the cytoplasm of bacteria, through the pore proteins in their outer membrane.[70]

In this study, we examined the anatomical characteristics of the secretory structures of *B. aracatubaensis*, *B. burchellii*, and *B. organensis* to determine the essential oil storage and pharmacobotanical characters of the taxon. *Baccharis* species are volatile oil producers and it is common to find glandular trichomes and secretory ducts in aerial vegetative organs,[6] as found in the species studied. Glandular trichomes and secretory ducts are secretory structures which produce and compartmentalize the volatile oils.[71] The biseriate and flagelliform types are the most common glandular trichomes present in *Baccharis*.[9] Considering the flagelliform glandular trichomes, besides their covering role, they may have secretory function, as observed in *Baccharis pentaptera* (Less.) DC.,[72] *Baccharis illinita* DC., *B. microdonta*, *B. pauciflosculosa*, *B. reticularioides*, and *B. sphenophylla*.[9]

In the present study, secretory ducts (*Figures 2A*, *2 C*, *2D*, *2F*, and *2G*) and flagelliform glandular trichomes (*Figures 2B*, *2E*) were observed in all the *Baccharis* specimens studied. Secretory ducts were found in the mesophyll (*Figures 2A*, *2G*) and midrib (*Figures 2C*, *2F*) of the leaves. They were composed of 6–20 cells with a uniseriate epithelium, dense cytoplasm, evident nucleus, and lipophilic content found next to the endodermis in the direction of the phloem (*Figures 2A*, *2C*, *2F*, *2G*). In the stem, the secretory ducts are found next to the endoderm that binds the internal part of the cortex (*Figure 2D*), and its walls are impregnated with lipophilic compounds. Additionally, three secretory ducts were found to meet near the phloem in the midrib of *B. organensis* (*Figure 2F*). The

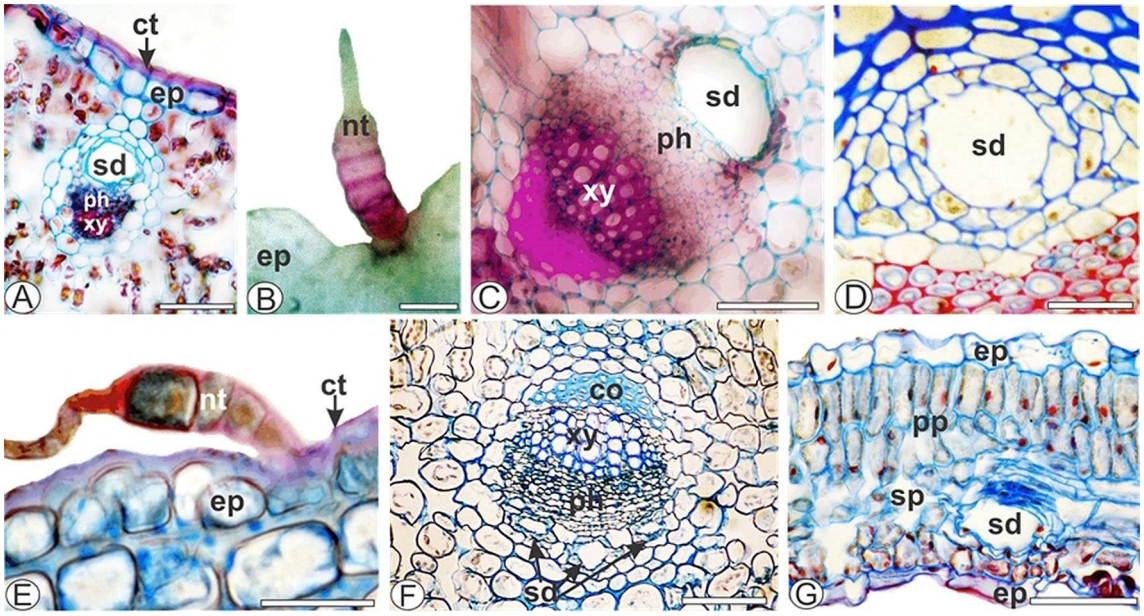

Figure 2. Aerial vegetative organs of *Baccharis aracatubaensis*, *B. burchellii*, and *B. organensis*. A), B), and C) – *Baccharis aracatubaensis*; D), E), F) – *Baccharis organensis*, and G) – *Baccharis burchellii*. A) Leaf in cross-section showing cuticle (cu), epidermis (ep), phloem (ph), secretory duct (sd), and xylem (xy). B) Frontal view of the leaf epidermis (ep) indicating the flagelliform glandular trichome (nt). C) Midrib of the leaf in cross-section showing phloem (ph), secretory duct (sd), and xylem (xy). D) Stem in cross-section indicating a secretory duct (sd). E) Stem in cross-section showing cuticle (cu), epidermis (ep), and flagelliform glandular trichome (gt). F) Midrib of the leaf in cross-section indicating collenchyma (co), phloem (ph), secretory ducts (sd), and xylem (xy). G). Leaf in cross-section indicating epidermis (ep), secretory duct (sd), spongy parenchyma (sp), and palisade parenchyma (pp). Scale bar = 50 μm (A, B, C, D, F, G), 12.5 μm (E).

presence of three secretory ducts in the midrib can be used to differentiate *B. organensis* from *B. aracatubaensis* and *B. burchellii*. This characteristic is not frequently observed in *Baccharis* species.[8,9] This characteristic may be helpful in quality control.

The flagelliform glandular trichomes were found in a small depression of the epidermis of the leaves (*Figure 2B*) and stems (*Figure 2E*). Oil droplets and secretory substances were observed visually under the microscope and also were confirmed by histochemical tests, in the body (*Figures 2B, 2E*), head cells as well as the tubular apical cells.

To the best of our knowledge, this is the first study that evaluates the chemical, antioxidant, and antimicrobial characteristics of the essential oils and anatomy of the aerial parts of female and male specimens from *B. aracatubaensis*, *B. burchellii*, and *B. organensis*, as part of the chemical, biological and anatomical studies of *Baccharis* species realized by our group of research.[73,74] The chemical composition of these essential oils is in agreement with the chemistry of the oils reported previously in another species from the genus *Baccharis*, with sesquiterpenes as a major component. The high antioxidant activity of the essential oils of *Baccharis* specimens studied suggests their potential as a natural source of biologically active compounds. The anatomical study of the aerial vegetative organs corroborates the findings from the studies of other *Baccharis* species. This is the first report regarding the collection and identification of female and male specimens from *B. burchellii* in Paraná State, Brazil. This study also confirms the importance of chemical and biological investigations of essential oils of *Baccharis* species (Asteraceae) in the search for new and safer bioactive agents. However, further investigations are necessary to confirm the potential of *Baccharis* essential oils as bioactive product useful for *in vivo* applications.

Experimental Section

Collection of Plant Material

Botanical material of male and female specimens of *Baccharis* were collected separately and randomly along a transect within the same population in November 2013 in the 'Morro do Canal', Municipality of Piraquara, Paraná State, Brazil. The inflorescences

were collected from *B. aracatubaensis* (25°30′52–48″ S/48°59′10–41″ O) and *B. organensis* (25°30′52–39″ S/48°59′10–78″ O) samples, at an elevation of 1200–1300 m. The inflorescences from *B. burchellii* samples were collected in proximity of one river (25°31′11–54″ S/49°00′21–17″ O) at an elevation of 906 m. The species were identified by botanists Osmar dos Santos Ribas, Dr. Gustavo Heiden and Dr. Angelo Alberto Schneider. The voucher specimens were deposited with the Botanical Museum of Curitiba (MBM), under the numbers: (MBM-286268/MBM-286267), (MBM-386275/MBM-386266), and (MBM-386257/MBM-386256), respectively.[75]

The access to the botanical material was authorized and licensed by the 'Ministério do Meio Ambiente – Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado' (SisGen) and registered under No. A7A6DCC.

Hydrodistillation of Essential Oils

The essential oil of the inflorescences from *B. aracatubaensis*, *B. burchellii*, and *B. organensis* was isolated by hydrodistillation using 100–150 g of each fresh plant material and 1.0–1.5 L of distilled water, for 3 h, using a Clevenger-type apparatus according to the method recommended in Brazilian Pharmacopeia 5th edn.[76] The distillated oil was extracted twice with ethyl ether and dried over anhydrous sodium sulfate, the excess solvent was withdrawn using nitrogen flow. The yield of each essential oil was calculated based on the weight of essential oil and the weight of the plant material, which was then stored in tightly closed dark vials at 4 °C until analysis.

GC/MS analyses were performed using a Shimadzu Model GC/MS-QP 2010 Plus apparatus equipped with an AOC-20i auto-injector, as per the method described by *Costa et al.* with some modifications.[50] The chromatograph was equipped with a ZB-5MS Phenomenex Zebron column (30×0.25 mm, 0.25 mm). The samples were injected at a 1:30 split ratio, and the analysis conditions were as follows: Sample concentration at 1:100 (μL) in CH_2Cl_2, injection volume of 1.0 μL, temperature of the injector 240 °C. The oven temperature was programmed to 40 °C/4 min, followed by increase with a rate of 4 °C/min to 240 °C, then by 10 °C/min up to 280 °C, and temperature of 280 °C/2 min; 60 min chromatographic run.

The retention indices for all the compounds were determined by injecting a standard solution containing the homologous series of *n*-alkanes (C_8–C_{18}). The calculation of peak area percentage was carried out by using the GC/MS Lab Solutions software (Shimadzu). Individual constituents were identified by the comparison of their mass spectra (MS) and retention indices (RI) with those reported in literature[37,38] and also in National Institute of Standards and Technology (NIST) mass spectral database.

Antioxidant Activity

$ORAC_{FL}$ Kinetic Assay. The antioxidant capacities of the essential oils of *B. aracatubaensis*, *B. burchellii*, and *B. organensis* were assessed through the oxygen radical absorbance capacity (ORAC) assay. The automated ORAC assay was performed on a Synergy 2 (Biotek, Winooski, VT) multidetection microplate reader system. The procedure was carried out according to the method already established by *Ou et al.*[52] with modifications and *Costa et al.*[51] The results were calculated using the differences of areas under the fluorescein (FL) decay curves between the blank and a sample and were expressed as mean (% RSD, relative standard deviation) of triplicate assays in micromole of Trolox equivalents (TE) per gram (μmol of TE g^{-1}) for essential oils tested and as relative Trolox equivalent (RTE) for standardized samples (Sigma-Aldrich) used as positive experimental controls.

TLC Autographic Assay for DPPH Radical Scavenging. The DPPH test allows the evaluation of hijacking the free radical, the purple coloration, by action of an antioxidant species present in the sample, by undergoing reduction to form yellow colored 2,2-diphenyl-1-picrylhydrazine (DPPH–H).[77] The procedure was carried out according to the method described by *Costa et al.*,[50] and the interpretation of results was performed as described by *Takamatsu et al.*[78] The samples that presented antioxidant activity produced yellowish spots and were classified according to the intensity of the yellow spots produced.

Samples with strong or high antioxidant activity presented an intense bright yellow spot, samples with medium antioxidant activity presented with a light yellow stain, samples with weak antioxidant activity showed a weak yellow stain or samples without antioxidant activity where devoid of yellowish coloration.[78]

Antimicrobial Assay

The antimicrobial assay of the essential oils (male and female specimens) from *B. organensis*, *B. burchellii*, and *B. aracatubaensis* was performed using Clinical and Laboratory Standards Institute (CSLI) microdilution method.[79] The oils were carried out against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8738), *Pseudomonas aeruginosa* (ATCC 9027), and *Candida albicans* (ATCC 10231).

The microorganism suspensions used for inoculation were prepared at 10^5 CFU (colony forming unit/mL) by diluting fresh cultures at McFarland 0.5 density. As positive controls were used 100 $\mu g\,mL^{-1}$ of chloramphenicol for the bacteria and 500 $\mu g\,mL^{-1}$ of ketoconazole for the yeast. The essential oils were solubilized with 5% of DMSO and H_2O, to adjust the concentrations to 100 $\mu g\,mL^{-1}$. The minimal inhibitory concentration (MIC) was defined as the lowest extract concentration showing no visible bacterial growth after incubation time, and the absorbance was measured in spectrophotometer to 540 nm.

Anatomical Study

At least six samples of mature leaves of *B. aracatubaensis*, *B. burchellii*, and *B. organensis* (male and female) were obtained from the sixth node and below, as well as stem fragments 5 to 15 cm from the shoot were collected to anatomical analyses. The plant materials were fixed in FAA 70[80] and kept in 70% ethanol solution.[81] These materials were sectioned by hand or dehydrated, embedded in glycol methacrylate (Leica Historesin®) and sectioned using the Leica RM-2145 microtome. Transverse and longitudinal sections were stained with astra blue and basic fuchsine combination.[82] The samples were clarified in frontal view of the epidermis.[83] Moreover, in order to show where the lipophilic compounds were located, Sudan III was used.[84] The photomicrographs were captured by an Olympus CX31 light microscope equipped with a C7070 control unit.

Statistical Analysis

The essential oil composition of the specimens was analyzed by hierarchical cluster analysis (HCA). The data were transformed with Log10 and the Ward's method as the amalgamation rule and Euclidean distances as metric were used to generate a dendrogram for the samples.

The analysis of the results obtained in the antioxidant assay was presented as mean (% RSD, relative standard deviation). Comparisons of the groups were evaluated by analyses of variance (one-way ANOVA), followed by the Tukey's post-hoc test. Differences were considered to be significant at $P < 0.001$.

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Author Contribution Statement

T.Z. and F.R.C. wrote the article. T.Z., J.B., and A.V.F.L. conducted fieldwork and collection of the samples, besides analyzed and prepared the data for the manuscript. G.H. identified the plant samples. A.V. and F.S.M. guided the antimicrobial assays. M.J.S. and J.V.N.M. guided the antioxidant assays and contributed to the analysis of the data. J.M.B. and P.A.R. performed the micromorphology experiments, evaluated, and analyzed the anatomy data. E.B., T.Z., and F.R.C. performed the statistical analysis.

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