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Diuretic and saluretic effects of *Baccharis dracunculifolia* DC. (Asteraceae)

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ABSTRACT

Ethnopharmacological relevance: Baccharis dracunculifolia DC. (Asteraceae) is an aromatic and medicinal species that is native to Brazil and other South American countries. It is popularly known as "*alecrim-do-campo*", "*alecrim-de-vassoura*", and "*vassourinha*", being used as infusions for various ailments. Moreover, in Brazil, this species is commonly utilized for treating cardiovascular disorders. Despite its common use , there is no evidence in the literature demonstrating the effects of *B. dracunculifolia* leaves on the cardiovascular system.

Aim of the study: We aimed to investigate the cardiorenal properties of the aqueous extract (ESBD) and essential oil (EOBD) of *B. dracunculifolia* aerial parts on Wistar rats.

Materials and methods: First, the plant material was collected and identified, its essential oil (EOBD) was extracted by hydro-distillation, while an ethanolic fraction was prepared after the obtention of the aqueous extract by infusion (ESBD). The chemical compositions of EOBD and ESBD were determined by GC-MS and LC-DAD-HRMS, respectively. The acute toxicity test was performed on female Wistar rats to determine the toxic effects after a single administration. Finally, the potential diuretic and hypotensive effects of ESBD and EOBD (doses at 30, 100, and 300 mg/kg) were evaluated on normotensive male Wistar rats.

Results: Thirty-eight compounds were annotated in EOBD by GC-MS (thirty-one identified), which was mainly composed by monoterpene hydrocarbons (81.9 %), highlighting α-pinene (14.2 %), β-pinene (39.8 %), and limonene (21.3 %). LC-DAD-HRMS analysis from ESBD revealed twenty-three compounds, including flavonoids (*C*-glycosylated flavones, *O*-glycosylated flavonols, and an aglycone flavanonol), and phenolic acid derivatives such as caffeoylquinic (chlorogenic), coumaroylquinic, and feruloylquinic acids. No signs of toxicity were observed after acute treatment with ESBD and EOBD, and it was suggested that the oral median lethal dose (LD_{50}) of the extracts is above 2000 mg/kg. In addition, a 7-day oral treatment with ESBD at a dose of 300 mg/kg was able to significantly increase urinary volume (thus, acting as diuretics) and renal excretion of sodium and chloride (saluretic action).

Conclusions: The findings of this study showed that a fraction derived from of the ethanol-soluble fraction of the aqueous extract (ESBD) and essential oil (EOBD) of *B. dracunculifolia* leaves is safe for acute administration on

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female Wistar rats. Additionally, oral treatment with ESBD (dose at 300 mg/kg) showed potential diuretic and saluretic effects in normotensive male Wistar rats.

1. Introduction

For thousands of years, people have been using medicinal plants as an alternative therapy to treat various ailments. The use of natural products derived from plants and microorganisms has been increasing in popularity globally (Dutra et al., 2016). In this context, Brazil has the highest number of plant species in the world, with 52,837 records to date, including those native, naturalized and cultivated (Flora e Funga do Brasil, 2024). Despite this huge number, the Brazilian Herbal Medicine only licensed as active medicinal plants less than 0.20 % (101 species, including 39 natives) (Carvalho et al., 2018). Therefore, the pharmacological research on Brazilian medicinal plants is of the utmost importance to valorize such biodiversity (Dutra et al., 2016).

The *Baccharis* L. genus is one of the largest and most diverse genera of aromatic and medicinal plants in the Asteraceae family, with 442 species distributed across South, Central and North America (Antunes et al., 2024). *Baccharis dracunculifolia* DC. (Asteraceae) is an aromatic and medicinal species native to Brazil, but also it is native to Argentina, Bolivia, Paraguay and Uruguay (Minteguiaga et al., 2021a; Armstrong et al., 2024). The species has a shrubby habit, reaching heights of 1–5 m, and it is widely distributed throughout the Brazilian territory, from the Southern to the Northeastern. In traditional medicine, *B. dracunculifolia* is known as "*alecrim-do-campo*", "*alecrim-de-vassoura*", and "*vassourinha*"; being used as infusions for various purposes such as anti-inflammatory, anti-ulcerative and to treat gastrointestinal and renal disorders (Minteguiaga et al., 2021a; Armstrong et al., 2024). Additionally, *B. dracunculifolia* is commonly used to treat cardiovascular diseases (Mendonça et al., 2020). Previous phytochemical studies have shown that essential oils, diterpenes, triterpenes, flavonoids, phenolic acids, and several specific glycosides named dracunculifosides are present in the aerial and underground organs of *B. dracunculifolia* (Minteguiaga et al., 2021a).

Metabolomics studies using liquid chromatography coupled to a diode array detector and tandem mass spectrometry (LC-DAD-HRMS) have been widely used due to their high accuracy, selectivity and sensitivity in detecting natural metabolites (Rafi et al., 2023). Furthermore, gas chromatography (GC) is highly suitable for the identification of volatile compounds, such as essential oils (Adams, 2017).

Although *B. dracunculifolia* has been extensively studied for its antioxidant, anti-inflammatory, and others properties (Armstrong et al., 2024), no previous studies have evaluated its potential cardiorenal or toxicological effects. Besides, the leaf infusion of *B. dracunculifolia* is used in traditional medicine to treat heart and kidney diseases, and there is no evidence reported to date regarding the chemical composition and efficacy of this extract being reported so far. Therefore, this study aimed to fill this gap by investigating thorough phytochemical, toxicological, and pharmacological investigation of *B. dracunculifolia* leaf extracts on Wistar rats after acute and prolonged exposure.

2. Material and methods

2.1. Chemicals

Xylazine and ketamine hydrochloride were purchased from Syntec (São Paulo, SP, Brazil), and heparin from Hipolabor (Belo Horizonte, MG, Brazil). Hydrochlorothiazide (HCTZ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents employed were of analytical grade, the solvents were of HPLC grade, while the water was ultrapure.

2.2. Plant material and identification

Fresh samples of flowering aerial parts of *B. dracunculifolia* DC. were collected from open and sunny habitats in Ponta Grossa, Paraná, Brazil (coordinates 25°05′26″ S, 50°06′17″ W, 907 m) in September 2020. The botanical material was identified by the taxonomist Dr. Gustavo Heiden and deposited in the Herbarium of the ECT, Pelotas, Rio Grande do Sul, Brazil (under the number ECT0008997). Access to the botanical source was authorized and licensed by the CGen/SisGen registered under the code AB29E78.

2.3. Extract preparation

After collection, the plant material was stabilized and dried in an oven at 30 °C. Afterwards, the samples were subjected to two types of extraction. 1) Dried plant material (100 g) was extracted by hydrodistillation for 3 h, in triplicate, using a Clevenger-type apparatus. The essential oil obtained from *B. dracunculifolia* (EOBD) had a yield of 0.6 % and was stored in amber glass vials with Teflon-sealed caps and refrigerated (−4 °C) for further analyses; and 2) Dried plant material (100 g) was extracted by infusion in 1 L of boiling water (93 °C). An aliquot of the infusion (200 mL), cooled to room temperature, was filtered and treated with three volumes of ethanol (600 mL), resulting both, in a precipitate and an ethanol-soluble fraction from *B. dracunculifolia* (ESBD). This procedure is important for precipitating primary metabolites that are not of interest for this study. The latter was concentrated using rotary evaporation (49 °C and 85 rpm), and then freeze-dried using an LD1500A equipment (Terroni/Lyotech, São Carlos, SP, Brazil), which had a yield of 9.45 %. The ESBD was stored in a freezer at −20 °C until analysis.

2.4. Chemical composition of EOBD by GC-MS

The essential oil EOBD was analyzed by GC-MS using a gas chromatograph coupled to a mass spectrometer Shimadzu QP2010 (single quadrupole), provided with an AOC-20i auto-injector and an electron ionization source (70 eV; Shimadzu, Kyoto, Japan). In the oven, it was attached a Rtx-5MS capillary column (dimensions: 30 m × 0.25 mm × 0.25 μm; Restek, Bellefonte, PA, USA). The oven temperature gradient program was as follows: 60 °C–320 °C at 4 °C/min, and finally the temperature was maintained to 320 °C for 20 min; temperature of the injection port and interface: 320 °C. The EOBD was prepared at 1 mg/mL in *n*-hexane and injected in the chromatographic system in split mode (1:5). Helium was used as carrier gas at a flow of 1.0 mL/min. The spectra were acquired in the scan mode, with a range set to 35–400 m/z. The identification of the components was based on the comparison with data of commercial mass spectral libraries (Mc Lafferty, 2000; Adams, 2017; Mondello, 2008; Wiley/NIST, 2023), and by the calculation of the linear retention indices (LRI) after the injection and analysis of a C_8-C_{20} *n*-alkane standard solution (Sigma-Aldrich) in the same analytical conditions as the samples (Minteguiaga et al., 2021b). LRIs were compared to the data reported in the literature.

2.5. Chemical composition of ESBD by LC-DAD-HRMS

The ethanol-soluble fraction from *B. dracunculifolia* (ESBD) was analyzed in an UFLC-20AD Shimadzu chromatographic system, coupled both to a diode array detector (DAD) and a Bruker Daltonics microTOF-Q III high resolution mass spectrometer (Bruker Co., Billerica, MA, USA). The sample was prepared at a concentration of 3 mg/mL in a methanol-water mixture (6:4 v/v), and filtered through syringe filters (PTFE

membrane, 0.22 μm × 13 mm; Millex, Merck Millipore, Darmstadt, Germany). Chromatographic separation was achieved using a Kinetex C-18 column (dimensions: 2.6 μm, 150 mm × 2.2 mm; Phenomenex, Torrance, CA, USA), which was maintained at 50 °C during the analyses. Ultrapure water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) were used as the mobile phases. The gradient elution profile was as follows: 0–2 min at 3 % B, 2–25 min from 3 % to 25 % B, and 25–40 min from 25 % to 80 % B. The sample (3 μL) was injected into the loop of the system, and the analyses were conducted in both negative and positive ion modes, with capillary voltages of 4.5 kV and 2.5 kV, respectively. The peak annotation was according to literature reports (see Results and Discussion section), while to confirm the chlorogenic acid's identity, authentic standards of 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid were co-injected (both purchased from Sigma-Aldrich).

2.6. Acute toxicity assessment

The acute toxicity tests of ESBD and EOBD were conducted on female Wistar rats (8–12 weeks old) following the protocol No. 425 established by the Organisation for Economic Cooperation and Development (OECD, 2022). ESBD (2000 mg/kg) was prepared in filtered water, while EOBD (2000 mg/kg) was emulsified in filtered water containing 2 % DMSO. Each solution was administered via oral gavage to different groups of rats (n = 5 per group) at a volume of 1 mL/100 g. The control group received the vehicle (filtered water) at a dose of 1 mL/100 g. Food was provided to the rats only after 3 h of the treatments. The animals were closely monitored for signs of toxicity or death during the first 24 h, and then for the following 14 days. Daily evaluations and recordings were made of body weight, food intake, and animal behavior using the "Hippocratic screening" method described by Malone and Robichaud (1962).

On day 15 after treatment, the animals were euthanized using isoflurane inhalation. The vital (heart, lung, liver, kidneys, and spleen) and reproductive (ovaries and uterus) organs were removed, cleaned, weighed, examined macroscopically, and the relative organ weight was calculated. Organ samples were sent for histopathological analysis, which was performed by veterinary pathologists from the UFGD.

2.7. Pharmacological investigations

2.7.1. Diuretic activity

The diuretic investigations of ESBD and EOBD were conducted following a methodology described by Gasparotto Junior et al. (2009). Male Wistar rats were randomly divided into eight experimental groups (n = 6) and were treated with ESBD, EOBD (doses at 30, 100, and 300 mg/kg), HCTZ (25 mg/kg), or the vehicle (filtered water 1 mL/100 g) by oral gavage once daily for 7 consecutive days. ESBD (2000 mg/kg) was prepared in filtered water, while EOBD (2000 mg/kg) was emulsified in filtered water containing 2 % DMSO. Each solution was administered via oral gavage to different groups of rats (n = 5 per group) at a volume of 1 mL/100 g. Each animal was placed in metabolic cages with free access to commercial feed and filtered water throughout the experiment. The total amount of urine was collected every 24 h and expressed as mL/100 g of body weight. Urinary levels of potassium (K^+), sodium (Na^+), and chloride (Cl^-) were measured using an ion-selective meter (COBAS INTEGRA 400 plus; Roche, Basel, Switzerland). Urinary excretion load of K^+, Na^+, and Cl^- was obtained by multiplying the concentration of electrolytes (mEq/L) by the urinary flow (mL/min). Data are expressed as μEq/min/100 g. Density and pH were determined in fresh urine samples using a handheld refractometer (NO107; Nova Instruments, Piracicaba, SP, Brazil) and a digital pH meter (Q400MT; Quimis Instruments, Diadema, SP, Brazil).

2.7.2. Blood pressure and heart rate evaluation

Blood pressure and heart rate parameters were evaluated as previously described by Gasparotto Junior et al. (2011) and Tolouei et al. (2019). Different groups of rats (n = 6) received a 7-day oral treatment with ESBD, EOBD (doses at 30, 100, and 300 mg/kg), HCTZ (25 mg/kg), or the vehicle (filtered water 1 mL/100 g). The animals were then intramuscularly anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg). A single bolus dose of heparin (50 UI) was administered subcutaneously. The left carotid artery was isolated, cannulated, and connected to a pressure transducer linked to a computerized recording system (PowerLab®), and an application program (Chart, v 7.00; both from ADI Instruments; Castle Hill, Australia) recorded systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) for 20 min.

All animal experiments were approved by the Ethics Committee in Animal Experimentation (CEUA) at the UFGD (protocol: 07/2020).

2.8. Statistical analyses

The data were analyzed to check for normal distribution and homogeneity of variance. The statistical analyses were conducted using one- or two-way analysis of variance (ANOVA), followed by Dunnett's test or the Student t-test, when applicable. The results were presented as mean ± standard error of the mean, with a significance level set at 95 % ($p < 0.05$). All the analyses and graphs were conducted using GraphPad Prism 9.0 (GraphPad Software, Boston, MA, USA).

3. Results and discussion

3.1. Chemical composition of ESBD and EOBD

The essential oil EOBD was analyzed by GC-MS (Fig. S1), and its components are listed in Table 1. The annotation/identification of the constituents was based on comparison of the spectral data obtained experimentally with those stored in commercial mass data libraries, and by comparing the experimental LRIs with those reported in the literature, even including studies on *B. dracunculifolia* volatile profile (Adams, 2017; Minteguiaga et al., 2018; Minteguiaga et al., 2021b).

Thirty-eight compounds were detected in EOBD, of which thirty-one were identified, including monoterpenes and sesquiterpenes. The predominant metabolites class identified were non-oxygenated (hydrocarbons) monoterpenes (81.9 %), from which the main ones were *α*-pinene (14.2 % of abundance, hereafter), *β*-pinene (39.8 %), and limonene (21.3 %). Several sesquiterpenes were also identified, such as *trans*-nerolidol (3.6 %) and spathulenol (2.2 %). All the compounds listed were already described in the literature for this species as shown in Table 1 (Loayza et al., 1995; Minteguiaga et al., 2018, 2021b).

When comparing EOBD composition results with those previously published on *B. dracunculifolia* essential oil from Brazilian origin, our sample demonstrated a low-abundance (3.6 %) of the chemo-marker *trans*-nerolidol (Minteguiaga et al., 2021a). However, Besten et al. (2015) informed previously that oils obtained from some individuals growing at the same collection place as ours (Ponta Grossa, Paraná State, Brazil) reached up to 44.36 % of abundance of this compound, while for other individuals of the same population it rendered only 4.51 %. Furthermore, Florão et al. (2012) collected *B. dracunculifolia* at the same place, and they did not detect *trans*-nerolidol in its essential oil. In the other hand, and as by the knowledge of the authors (Armstrong et al., 2024), the EOBD essential oil informed here is the more concentrated one in *β*-pinene (39.8 %) reported in the literature to date for Brazilian samples, being the second so-concentrated (29.8 %) one of those informed by Tomazzoli et al. (2021) for a vegetal population of the same collection region as ours (Paraná State). Outside Brazil, Frizzo et al. (2008) reported a *B. dracunculifolia* essential oil containing 43.4 % of *β*-pinene in the Southern Uruguay region. The high difference in composition is not only given by the geographical site and individuality, but also due to the influence of the climatic conditions in every year, seasonality inside a year, biotic interactions and plant sexuality (a key

Table 1
Components annotated and identified by GC-MS from the essential oil EOBD of *B. dracucunlifolia*.

	Components	LRI_{Exp}	LRI_{Lit}	Relative content (abundance) [%]
	Monoterpene hydrocarbons			81.71 %
1	α-thujene[1,2,5,6,7]	923	924	0.14
2	α-pinene[1,2,5,6,7]	928	932	14.24
3	Camphene[2,6]	941	946	0.20
4	Sabinene[1,2,7]	972	969	0.54
5	β-pinene[1,2,4,5,6,7]	973	974	39.84
6	Myrcene[1,2,4,5,6,7]	994	988	4.14
7	*p*-cymene[1,2,5,6]	1029	1020	0.27
8	Limonene[1,2,4,5,6,7]	1033	1024	21.30
9	*trans*-β-ocimene[1,2,4,5,6,7]	1053	1044	0.58
10	γ-terpinene[1,2,6]	1064	1054	0.22
11	α-terpinolene[1,2,6]	1091	1086	0.24
Oxygenated Monoterpenes				**3.94 %**
12	Linalool[1,2,4,5,6]	1101	1095	0.25
13	*endo*-fenchol[6]	1117	1114	0.06
14	*trans*-pinocarveol[5,6]	1143	1135	0.90
15	Pinocarvone[5,6]	1167	1160	0.27
16	Terpinen-4-ol[1,2,5,6,7]	1181	1174	0.57
17	α-terpineol[1,2,4,5,6,7]	1193	1186	1.01
18	Myrtenal[5,6]	1198	1195	0.88
Sesquiterpene hydrocarbons				**3.99 %**
19	β-elemene[1,2,5,6,7]	1393	1389	0.08
20	*trans*-β-caryophyllene[1,2,4,5,6,7]	1421	1417	1.08
21	*allo*-aromadendrene[1,2,4,6]	1452	1458	0.21
22	α-humulene[1,2,4,5,6]	1456	1452	0.11
23	Germacrene D[1,2,4,5,6,7]	1483	1484	0.44
24	Ledene[2,6]	1485	1496	0.11
25	Bicyclogermacrene[1,2,4,5,7]	1497	1500	1.43
26	α-muurolene[1,2,6,7]	1502	1500	0.07
27	δ-cadinene[1,2,4,6,7]	1526	1522	0.46
Oxygenated Sequiterpenes				**6.62 %**
28	*trans*-nerolidol[1,2,4,5,7]	1567	1561	3.59
29	Spathulenol[1,2,3,4,5,6,7]	1579	1577	2.21
30	Viridiflorol[2,4,6,7]	1585	1592	0.74
31	τ-cadinol[1,2,5,6]	1635	1638	0.08
Total identified				**96.26 %**
Non-identified				**3.74 %**
32	unknown 1	977		0.76
33	unknown 2 (tentative: δ-2-carene)	1020		0.18
34	unknown 3	1180		0.35
35	unknown 4	1485		0.80
36	unknown 5	1495		0.53
37	unknown 6	1585		0.57
38	unknown 7	1592		0.55

aspect for dioic species like *B. dracunculifolia*) (Besten et al., 2015; Minteguiaga et al., 2021b).

NI: non-identified; LRI_{Exp}: retention indices of the components determined experimentally in this work; LRI_{Lit}: retention indices reported by Adams (2017). Components reported for *B. dracunculifolia* previously by: (1) Loayza et al. (1995), (2) Frizzo et al. (2008), (3) Florão et al. (2012), (4) Besten et al. (2015), (5) Minteguiaga et al. (2018), (6) Minteguiaga et al., (2021b), (7) Tomazzoli et al. (2021).

The chemical constituents from the ethanol fraction of *B. dracunculifolia* (ESBD) were analyzed by LC-DAD-HRMS, and its constituents were annotated (Fig. S2, Table 2). The molecular formula was determined by accurate MS data, considering errors and mSigma up to 8 ppm and 20 ppm, respectively. The spectral data, including UV, MS, and MS/MS were compared to the literature. The annotated compounds were some flavonoids, including flavones (apigenin glycosides and *O*-methyl luteolin), flavonols (quercetin glycosides), and a flavanonol (dihydrokaempferide); as well as phenolic acid derivatives, such as caffeoylquinic (chlorogenic), coumaroylquinic, and feruloylquinic acids.

The chlorogenic acids exhibited UV spectra similar to the chromophores of caffeic acid/ferulic acid at $\lambda_{max} \approx 294$ and 324 nm (peaks **2**, **4**, **9**, **17**, **18**, and **19**, hereafter) and coumaric acid at $\lambda_{max} \approx 299$ and 310 nm (peak **5**) (Klider et al., 2024). For the annotation of these metabolites, the spectral data were compared with the described by Clifford et al. (2003, 2005), which are mainly based on the fragment ions at *m/z* 191 [quinic acid-H]$^-$, *m/z* 179 [caffeic acid-H]$^-$, *m/z* 173 [quinic acid--H-H_2O]$^-$. The relative intensities of these ions were useful to suggest the annotation of the metabolites and, for some of them, the authentic standards were co-injected to confirm, such as 3-*O*-caffeoylquinic acid (**2**) and 4-*O*-caffeoylquinic acid (**4**).

For the flavonoid annotation, the fragmentation patterns and UV spectra provide crucial insights into their structural characteristics. The peaks **8**, **10**, **11**, **13**, and **21** showed UV spectra compatible to flavones ($\lambda_{max} \approx 275$ and 330 nm), while **14**, **15**, and **16** were relative to flavonols ($\lambda_{max} \approx 270$ and 350 nm) (Klider et al., 2024), and the metabolite of the peak **20** with a flavanonol ($\lambda_{max} \approx 285$ nm) (Pan et al., 2010).

The MS/MS of flavones of **8**, **10**, **11**, and **13** showed successive losses of water molecules and relative to *C*-hexosyl of 90 and 120 u and *C*-pentosyl groups of 60 and 90 u (Cao et al., 2014), for example the observed for apigenin 6,8-di-*C*-hexoside (vicenin-2) (**8**), and *C*-pentosyl *C*-hexosyl apigenin (**10**, **11**, and **13**). The compound **21** was a methylated flavone annotated as *O*-methyl luteolin, which the presence of the molecular ion *m/z* 299 and the main fragment *m/z* 284 (loss of 15 u ($CH_3^{\bullet}$) were compatible to this compound (aglycone) (Klider et al., 2024).

The peaks **14**–**16** exhibited losses of 162, 146, and 176 u in the MS/MS, being annotated as *O*-glycosylated flavonols with hexosyl, deoxyhexosyl, and glucuronyl motifs, respectively (Carazzone et al., 2013; Klider et al., 2024). Additionally, these metabolites revealed the same fragment ion relative to the aglycone quercetin (*m/z* 300/301). In addition, the metabolite of the peak **20** (*m/z* 301.0692 [M-H]$^-$) revealed a fragment ion at *m/z* 283, yielded by the loss of a water molecule, which is compatible to dihydrokaempferide (Finger et al., 2013).

Some studies in the literature have conducted analyses of the aqueous/organic *B. dracunculifolia* extracts by LC-DAD-HRMS (or other LC analytical set-ups, including MS), i.e.: Bonin et al. (2020), Rodrigues et al. (2020), Veiga et al. (2023), and Batista et al. (2024). Beyond other terpenic components, Bonin et al. (2020) reported two flavanones (naringenin and apigenin) and two flavonols (kaempferol, kaempferide) in the hydroalcoholic extract of *B. dracunculifolia* plant material. Rodrigues et al. (2020) reported 7-*O*-methylkaempferol and, in agreement with the present work, vicenin 2 (6,8-di-*C*-glucosylapigenin) in the leaves' hydroalcoholic extract of the same species. Veiga et al. (2023) informed the presence of kaempferol/kaempferide, pinocembrin (a flavanone) and betuletol (a flavone) in the ethanolic extract from the leaf buds of *B. dracunculifolia*. Finally, Batista et al. (2024) found the flavonols quercetin and kaempferol, and the flavanonol isosakuranetin in the ethanolic macerate of the leaves. As it can be seen, in all the described studies, flavonoids were found as relevant components, but generally aglycons are more frequently reported than glycosides. In the current work, by using an infusion with boiling water of the plant material as the first step for obtaining ESBD, the resulting extract was more concentrated in glycosides as compared to the literature reports, which in general employed hydroalcoholic or ethanolic extract of the plant parts. In the other hand, phenolic acids derived from caffeic (with emphasis on chlorogenic acids), coumaric and ferulic acids as those reported in Table 2 are frequent components of the polar extract of the plant (Rodrigues et al., 2020; Minteguiaga et al., 2021a; Veiga et al., 2023).

3.2. Safety evaluation

The use of plant species in traditional medicine has become

Table 2
Annotated components of the ethanol-soluble fraction of *B. dracunculifolia* (ESBD) by LC-DAD-HRMS.

Peak	RT (min)	Compound	MF	UV (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$(*m/z*)	MS/MS Negative mode
1	1.2	Di-*O*-hexoside	$C_{12}H_{22}O_{11}$	–	–	341.1089	
2	6.6	3-*O*-caffeoylquinic acid*	$C_{16}H_{18}O_9$	293, 322	355.1024	353.0878	161, 191, 179, 173
3	8.3	NI	$C_{14}H_{20}O_8$	288	317.1231	315.1085	195, 191, 179, 165, 151
4	11.1	4-*O*-caffeoylquinic acid*	$C_{16}H_{18}O_9$	294, 324	377.0843^{Na}	353.0878	191, 173
5	13.8	*O*-coumaroylquinic acid	$C_{16}H_{18}O_8$	299, 310	–	337.0907	191, 163
6	14.1	NI	$C_{18}H_{22}O_{11}$	–	–	413.1071	381, 371, 249
7	14.8	NI	$C_{17}H_{22}O_{10}$	293, 328	409.1105^{Na}	385.1140	367, 267, 249, 191, 173, 161
8	15.1	Apigenin 6,8-di-*C*-hexoside (vicenin-2)	$C_{27}H_{30}O_{15}$	270, 333	595.1657	593.1453	503, 473, 353
9	15.5	*O*-feruloylquinic acid	$C_{17}H_{20}O_9$	299, 321	369.1180	367.1035	191, 173
10	16.4	*C*-pentosyl *C*-hexosyl apigenin	$C_{26}H_{28}O_{14}$	270, 335	565.1552	563.1348	473, 443, 413, 383, 353
11	16.8	*C*-pentosyl *C*-hexosyl apigenin	$C_{26}H_{28}O_{14}$	272, 334	565.1556	563.1369	503, 473, 443, 413, 383, 353
12	17.5	NI	$C_{22}H_{32}O_{14}$	285, 335(sh)	521.1865	519. 1719	399, 309, 279, 267, 237
13	17.9	*C*-pentosyl *C*-hexosyl apigenin	$C_{26}H_{28}O_{14}$	271, 331	565.1552	563.1348	473, 443, 383, 353
14	18.9	*O*-hexosyl-deoxyhexosyl quercetin	$C_{27}H_{30}O_{16}$	262, 352	611.1607	609.1402	477, 301
15	18.9	*O*-glucuronyl quercetin	$C_{21}H_{18}O_{13}$	265, 350	479.0807	477.0656	301, 179
16	19.2	*O*-hexosyl quercetin	$C_{21}H_{20}O_{12}$	265, 350	465.1030	463.0886	300, 179
17	20.3	3,4-di-*O*-*E*-caffeoylquinic acid*	$C_{25}H_{24}O_{12}$	297, 324	517.1341	515.1195	353, 191, 179, 173
18	21.0	3,5-di-*O*-*E*-caffeoylquinic acid*	$C_{25}H_{24}O_{12}$	297, 324	517.1341	515.1195	353, 191, 179, 173
19	22.6	4,5-di-*O*-*E*-caffeoylquinic acid*	$C_{25}H_{24}O_{12}$	297, 324	517.1341	515.1195	353, 191, 179, 173
20	26.1	Dihydrokaempferide	$C_{16}H_{14}O_6$	285	303.0871	301.0692	283, 173, 151
21	30.0	*O*-methyl luteolin	$C_{16}H_{12}O_6$	273, 336	301.0707	299. 0561	284, 256, 255, 227, 183
22	32.8	NI	$C_{20}H_{28}O_5$	290	349.2024	347.1864	317, 299, 273, 255
23	35.5	NI	$C_{20}H_{26}O_4$	290	331.1900	329.1748	299, 255

NI: non-identified; RT: retention time; MF: molecular formula; ^{Na}: $[M+Na]^+$ adduct; sh: shoulder.

increasingly popular because of the belief that everything natural is safe. However, plants have the same potential to cause harm to human and animal health as other conventional medicines (Tolouei et al., 2019). Therefore, an acute toxicity test of ESBD and EOBD was conducted before moving on to effectiveness evaluations.

A single dose of ESBD or EOBD did not result in any deaths or significant changes in behavior during the 14-day observation period. There were no significant differences in final body weight gain or daily feed intake compared to the control group (Table S3). Relative organ weight (heart, lung, liver, kidneys, spleen, ovaries, and uterus) did not show any significant variations in all the treated animals (with ESBD or EOBD) compared to the control group. Macroscopic and histological evaluations did not reveal any signs of toxicity. It is important to note that animals experiencing toxic effects typically exhibit behavioral changes, reduced feed and water consumption, leading to weight loss. Furthermore, organ weight is a key indicator of the physiological and pathological condition of the animals (Sellers et al., 2007). The results of this study suggest that the oral median lethal dose (LD_{50}) of the *B. dracunculifolia* EOBD and ESBD extracts is above 2000 mg/kg.

Toxicological studies have previously been conducted on different species of *Baccharis*. An orally administered tincture of *B. trimera* (Less.) DC. at a single dose of 2000 mg/kg did not result in any significant hematological or biochemical changes, signs of toxicity, or mortality in Wistar rats (da Silva et al., 2016). In a similar experimental model in Wistar rats, with oral administration of ethanol soluble fraction (2000 mg/kg) of *B. milleflora* (Less.) DC., Klider et al. (2024) not observed changes in behavior, deaths, weight of organs, and abnormalities of organs in the animals. Another study found that the aqueous extract of *B. genistelloides* (Lam). Pers. at doses at 4.2 and 42 mg/kg, after 37 days of oral administration, did not show genotoxic effects on the kidney and liver, and did not cause weight alterations in the lungs, liver, and kidneys (Coelho et al., 2004). It is worth noting that certain species within the genus, such as *B. artemisioides* Hook. & Arn., *B. coridifolia* DC., and *B. megapotamica* Spreng., in association with some fungi produce macrocyclic trichothecenes, which are highly toxic substances (Fernandes et al., 2021). A study on *B. dracunculifolia*, conducted under different conditions from the current study, showed mild damage and other changes in liver histology in fish during *in vivo* tests. However, this study involved a 21-day feeding period with a very high dose of ethanolic extracts from *B. dracunculifolia*, similar to the quantity used in traditional medicine by humans (Oliveira-Lima et al., 2019). Other studies in the literature pointed out to the absence of mutagenic/genotoxic effects or cytotoxicity against Vero cells of the hydroalcoholic or organic extracts of *B. dracunculifolia* aerial parts (Resende et al., 2007; Da Silva Filho et al., 2009; Roberto et al., 2016; Bonin et al., 2020), thus confirming their safety.

3.3. Diuretic effects

Table 3 shows the effects of a 7-day oral treatment of male Wistar rats with ESBD on the overall urine volume and the excretion of K^+, Na^+, and Cl^-. The acute treatment with ESBD (doses at 30, 100, and 300 mg/kg) on day 1 did not increase urinary volume compared to the control group. However, on the third day, animals receiving ESBD at 300 mg/kg showed a significant increase in urinary excretion compared to the control rats. Additionally, on day 7, diuresis was significantly increased in rats treated with all the ESBD doses compared to control animals. The excretion of electrolytes was investigated on days 1, 3, and 7. Significant differences were observed in animals treated with ESBD or HCTZ compared to the control animals. All animals receiving ESBD at doses of 30, 100, or 300 mg/kg or HCTZ showed a significant increase in urinary excretion of Na^+ and Cl^- compared to control rats. Only HCTZ significantly increased K^+ excretion on days 3 and 7 compared to the control group.

Table 3 shows the effects of a 7-day oral treatment of male Wistar rats with EOBD on the urine volume and the renal excretion of K^+, Na^+, and Cl^-. The treatment with EOBD (doses at 30, 100, and 300 mg/kg) did not significantly affect urinary volume or renal electrolyte excretion throughout the experimental period (days 1–7). As expected, HCTZ increased urinary excretion on days 1 and 7, as well as the excretion of Na^+ and Cl^- on days 1–7, and K^+ on days 3 and 7. The pH and density values were not altered by any treatment (data not shown).

Diuretics have been found to be beneficial in managing hypervolemia and electrolyte imbalances and are recommended as the initial treatment for hypertension (Roush et al., 2014). Effective diuretics are those that increase the excretion of water and electrolytes by the kidneys (Roush et al., 2014). In this study, doses of ESBD at 300 mg/kg produced a sustained diuretic effect starting from the third day. Additionally, on the first day of treatment, ESBD (at doses of 30, 100, and 300 mg/kg) increased the urinary excretion of Na^+ and Cl^- in a dose-dependent

Table 3
Effects of prolonged oral administration to male Wistar rats of extracts obtained from the aerial parts of *B. dracunculifolia* on cumulative urine volume, as well as the excretion of Na^+, K^+, and Cl^-.

Group	Cumulative urine volume (mL/100 g)	ElNa$^+$ (μEq/min/100 g)	ElK$^+$ (μEq/min/100 g)	ElCl$^-$(μEq/min/100 g)
ESBD				
Day 1				
Control	8.20 ± 0.84	0.20 ± 0.01	0.11 ± 0.02	0.22 ± 0.01
HCTZ 25 mg/kg	12.13 ± 1.12*	0.41 ± 0.01*	0.14 ± 0.03	0.41 ± 0.01*
ESBD 30 mg/kg	6.42 ± 0.88	0.24 ± 0.01*	0.13 ± 0.02	0.25 ± 0.01*
ESBD 100 mg/kg	7.13 ± 0.62	0.28 ± 0.01*	0.15 ± 0.03	0.27 ± 0.01*
ESBD 300 mg/kg	8.13 ± 0.86	0.44 ± 0.01*	0.13 ± 0.02	0.44 ± 0.01*
Day 3				
Control	15.81 ± 2.23	0.20 ± 0.01	0.12 ± 0.02	0.21 ± 0.01
HCTZ 25 mg/kg	18.32 ± 2.33	0.44 ± 0.02*	0.21 ± 0.02*	0.44 ± 0.01*
ESBD 30 mg/kg	13.40 ± 1.39	0.24 ± 0.01*	0.13 ± 0.02	0.24 ± 0.01*
ESBD 100 mg/kg	16.07 ± 2.05	0.30 ± 0.02*	0.14 ± 0.02	0.30 ± 0.02*
ESBD 300 mg/kg	19.13 ± 2.23*	0.42 ± 0.01*	0.14 ± 0.02	0.43 ± 0.02*
Day 7				
Control	28.00 ± 4.20	0.21 ± 0.01	0.11 ± 0.02	0.20 ± 0.01
HCTZ 25 mg/kg	34.78 ± 3.18*	0.43 ± 0.01*	0.21 ± 0.03*	0.43 ± 0.00*
ESBD 30 mg/kg	28.33 ± 3.49	0.24 ± 0.01*	0.13 ± 0.02	0.24 ± 0.01*
ESBD 100 mg/kg	32.39 ± 4.84*	0.30 ± 0.01*	0.13 ± 0.02	0.30 ± 0.02*
ESBD 300 mg/kg	35.09 ± 2.48*	0.44 ± 0.01*	0.14 ± 0.03	0.44 ± 0.03*
EOBD				
Day 1				
Control	8.20 ± 0.84	0.20 ± 0.01	0.14 ± 0.03	0.22 ± 0.01
HCTZ 25 mg/kg	15.33 ± 1.21*	0.41 ± 0.01*	0.17 ± 0.02	0.41 ± 0.01*
EOBD 30 mg/kg	5.90 ± 0.33	0.19 ± 0.01	0.11 ± 0.02	0.20 ± 0.01
EOBD 100 mg/kg	7.59 ± 0.41	0.21 ± 0.01	0.11 ± 0.02	0.20 ± 0.02
EOBD 300 mg/kg	5.76 ± 0.27	0.21 ± 0.01	0.11 ± 0.02	0.23 ± 0.02
Day 3				
Control	15.81 ± 2.23	0.20 ± 0.01	0.09 ± 0.02	0.21 ± 0.01
HCTZ 25 mg/kg	18.32 ± 2.33	0.44 ± 0.02*	0.21 ± 0.00*	0.44 ± 0.01*
EOBD 30 mg/kg	13.86 ± 2.21	0.19 ± 0.01	0.10 ± 0.01	0.21 ± 0.01
EOBD 100 mg/kg	15.06 ± 1.69	0.23 ± 0.03	0.11 ± 0.02	0.21 ± 0.01
EOBD 300 mg/kg	13.23 ± 2.13	0.22 ± 0.02	0.12 ± 0.02	0.22 ± 0.01
Day 7				
Control	28.00 ± 4.20	0.21 ± 0.02	0.11 ± 0.02	0.20 ± 0.02
HCTZ 25 mg/kg	35.18 ± 2.23*	0.43 ± 0.03*	0.21 ± 0.01*	0.43 ± 0.04*
EOBD 30 mg/kg	22.42 ± 3.11	0.21 ± 0.02	0.10 ± 0.02	0.21 ± 0.02
EOBD 100 mg/kg	31.67 ± 1.69	0.22 ± 0.03	0.10 ± 0.02	0.21 ± 0.02
EOBD 300 mg/kg	25.06 ± 2.60	0.23 ± 0.02	0.13 ± 0.02	0.23 ± 0.03

Statistical analysis was performed using two-way ANOVA followed by Dunnett's test. Values are expressed as mean ± S.E.M. (n = 6). *p ≤ 0.05 when compared with the control group. El: Excreted load; HCTZ: normotensive rats treated with hydrochlorothiazide; Control: (vehicle-group normotensive rats); ESBD: normotensive rats treated with aqueous extract of *Baccharis dracunculifolia*; EOBD: normotensive rats treated with essential oil of *Baccharis dracunculifolia*.

manner (thus, acting as saluretics). The most effective dose was found to be ESBD at 300 mg/kg, as it produced similar results to HCTZ. One common side effect of HCTZ is hypokalemia (Roush et al., 2014). It is worth noting that the treatment with ESBD did not affect the renal elimination of potassium, which suggests a therapeutic advantage compared to the use of HCTZ. The diuretic and saluretic effects observed with ESBD may be attributed to the presence of flavonoids and chlorogenic acids. These metabolites are known to influence renal function by inhibiting Na^+/K^+-ATPase activity or modulating aquaporin expression, leading to increased water and sodium excretion (Vargas et al., 2018; Wei et al., 2022).

In another study with similar experimental model, Klider et al. (2024) observed that the extract of *B. milleflora* shown a significant diuretic and natriuretic effect, without affecting renal potassium elimination. In the knowledge of the authors, to date, there have been no *in vivo* studies examining the diuretic potential of *B. dracunculifolia*. Also in this context, plants extract rich in phenolics showed similar renal effects attributed to their flavonoid and phenolic acid content (Mariano et al., 2022; Souza et al., 2021), reinforcing the potential role of these compounds in diuretic action.

3.4. Effects on blood pressure and heart rate

The treatment for 7 days with ESBD and EOBD was not able to promote any significant changes in blood pressure or heart rate levels when compared to the control group (Table S4). It is important to note that many antihypertensive drugs do not significantly reduce blood pressure in normotensive individuals and tend to strengthen their antihypertensive response within 4 weeks after therapy begins (Oparil et al., 2018). Studies have demonstrated that certain natural compounds, such as flavonoids, exhibit diuretic and natriuretic effects in normotensive and hypertensive rats without significantly altering blood pressure or heart rate. These findings suggest a favorable cardiovascular safety profile and support the potential therapeutic use of such compounds as diuretics (Boeing et al., 2017; Mariano et al., 2020).

Although *B. dracunculifolia* is used in traditional medicine to treat heart and kidney diseases, a complete cardiovascular study of the extract has not yet been conducted. A small-scale human trial (n = 8) was reported in which *B. dracunculifolia* extract (20 mg/kg) had no effects on blood pressure and heart rate in healthy patients (Oliveira et al., 2014). Recently Batista et al. (2024) found that the ethanolic extract of the same species administered as a nano-emulsion (doses at 25–50 mg/kg) acted as antihypertensive in rats, research that also suggested the involvement of the neurogenic mechanisms on the action. Other studies have been reported for other *Baccharis* species, such as *Baccharis trimera*, whose ethanol-soluble fraction was able to significantly lower blood pressure levels (Souza et al., 2020; Mendes et al., 2021). Additionally, studies have been done on Brazilian green propolis (botanical origin: *B. dracunculifolia*), which showed hypotensive activity in spontaneously hypertensive rats (Mishima et al., 2005).

4. Conclusion

The results from this study showed that the fractions ESBD and EOBD of *B. dracunculifolia* leaves exhibited a chemical composition rich in flavonoid and phenolic acids (ESBD), and monoterpene hydrocarbons (EOBD). These fractions are safe for acute administration in female Wistar rats. Furthermore, oral treatment with ESBD (300 mg/kg) demonstrates diuretic and saluretic effects in normotensive male Wistar rats (EOBD did not demonstrate so similar effects). This is the first scientific report demonstrating the diuretic potential for the species *B. dracunculifolia*. However, further research is needed to investigate deeper the potential hypertensive effects of ESBD in hypertensive animals.

CRediT authorship contribution statement

Valter Paes de Almeida: Methodology, Investigation, Data curation. **Sara Emília Lima Tolouei:** Methodology, Investigation, Data curation. **Lislaine Maria Klider:** Methodology, Investigation, Data curation. **Aline Aparecida Macedo Marques:** Methodology, Investigation, Data curation. **Karyne Garcia Tafarelo Moreno:** Methodology, Formal analysis, Data curation. **Katyuce Souza Farias:** Methodology, Formal analysis, Data curation. **Izadora Bonfim:** Methodology, Formal analysis, Data curation. **Emerson Luiz Botelho Lourenço:** Project administration, Funding acquisition, Formal analysis. **Gustavo Heiden:** Methodology, Data curation. **Manuel Minteguiaga:** Methodology, Formal analysis, Data curation. **Paulo Vitor Farago:** Methodology, Formal analysis, Data curation. **Denise Brentan Silva:** Writing – review & editing, Writing – original draft. **Arquimedes Gasparotto Junior:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Jane Manfron:** Writing – review & editing, Writing – original draft, Supervision, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2025.120313.

Data availability

Data will be made available on request.

References

Adams, R.P., 2017. Identification of Volatile Oil Components by Gas Chromatography, Mass Spectrometry. Allure Publishing Corporation, Carol Stream, USA.

Antunes, K.A., Raman, V., Perera, W.H., Heiden, G., Pontarolo, R., Farago, P.V., Khan, I. A., Manfron, J., 2024. Authentication and quality control of the Brazilian traditional herb 'carquejas' (*baccharis* species) using morpho-anatomy and microscopy. Plants 13, 3030. https://doi.org/10.3390/plants13213030.

Armstrong, L., Raeski, P.A., de Almeida, V.P., Minteguiaga, M., Novatski, A., Raman, V., Gasparotto Junior, A., Naman, C.B., Manfron, J., 2024. *Baccharis dracunculifolia* DC. A review of research advances from 2004 to 2024, with new micromorphology and essential oil investigations. J. Herb. Med. 48, 100952. https://doi.org/10.1016/j.hermed.2024.100952.

Batista, M.A.C., Freitas, F.E.D.A., Braga, D.C.D.A., de Souza, J.A., Antunes, V.R., de Souza, G.H.B., dos Santos, O.D.H., Brandão, G.C., Kohlhoff, M., Ceron, C.S., de Moura, S.A.L., Cardoso, L.M., 2024. Antihypertensive effect of a nanoemulsion of *Baccharis dracunculifolia* leaves extract in sodium-dependent hypertensive rats. Nat. Prod. Res. 1–8. https://doi.org/10.1080/14786419.2024.2397724.

Besten, M.A., Nunes, D.S., Granato, D., Sens, S.L., Wisniewski, Edesio, L.S., Edesio, L.S., Riva-Scharf, D., 2015. Volatile components from galls induced by *Baccharopelma dracunculifoliae* (hemiptera: psyllidae) on leaves of *Baccharis dracunculifolia* (asteraceae). Quim. Nova 38, 66–70. https://doi.org/10.5935/0100-4042.20140288.

Boeing, T., Silva, L.M., Mariott, M., Andrade, S.F., Souza, P., 2017. Diuretic and natriuretic effect of luteolin in normotensive and hypertensive rats: role of muscarinic acetylcholine receptors. Pharmacol. Rep. 69, 1121–1124. https://doi.org/10.1016/j.pharep.2017.05.010.

Bonin, E., Carvalho, V.M., Avila, V.D., Aparecida dos Santos, N.C., Benassi-Zanqueta, É., Contreras Lancheros, C.A., Santos Previdelli, I.T., Ueda-Nakamura, T., Alves de Abreu Filho, B., Nunes do Prado, I., 2020. *Baccharis Dracunculifolia*: chemical constituents, cytotoxicity and antimicrobial activity. Lwt 120, 108920. https://doi.org/10.1016/j.lwt.2019.108920.

Cao, J., Yin, C., Qin, Y., Cheng, Z., Chen, D., 2014. Approach to the study of flavone di-*C*-glycosides by high performance liquid chromatography-tandem ion trap mass spectrometry and its application to characterization of flavonoid composition in *Viola yedoensis*. J. Mass Spectrom. 49, 1010–1024. https://doi.org/10.1002/jms.3413.

Carazzone, C., Mascherpa, D., Gazzani, G., Papetti, A., 2013. Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionisation tandem mass spectrometry. Food Chem. 138, 1062–1071. https://doi.org/10.1016/j.foodchem.2012.11.060.

Carvalho, A.C.B., Lana, T.N., Perfeito, J.P.S., Silveira, D., 2018. The Brazilian market of herbal medicinal products and the impacts of the new legislation on traditional medicines. J. Ethnopharmacol. 212, 29–35. https://doi.org/10.1016/j.jep.2017.09.040Choi.

Clifford, M.N., Knight, S., Kuhnert, N., 2005. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS^n. J. Agric. Food Chem. 53, 3821–3832. https://doi.org/10.1021/jf050046h.

Clifford, M.N., Johnston, K.L., Knight, S., Kuhnert, N., 2003. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. J. Agric. Food Chem. 51, 2900–2911. https://doi.org/10.1021/jf026187q.

Coelho, M.G.P., Reis, P.A., Gava, V.B., Marques, P.R., Gayer, C.R., Laranja, G.A.T., Felzenswalb, I., Sabino, K.C.C., 2004. Anti-arthritic effect and subacute toxicological evaluation of *baccharis Genistelloides* aqueous extract. Toxicol. Lett. 154, 69–80. https://doi.org/10.1016/j.toxlet.2004.07.004.

da Silva, A.R.H., Reginato, F.Z., Guex, C.G., Figueiredo, K.C., Araldi, I.C.C., Freitas, R.B., Boligon, A.A., Athayde, M.L., Mazzanti, C.M.A., Hübscher, G.H., Bauermann, L.F., 2016. Acute and sub-chronic (28 days) oral toxicity evaluation of tincture *Baccharis trimera* (less) backer in Male and female rodent animals. Regul. Toxicol. Pharmacol. 74, 170–177. https://doi.org/10.1016/j.yrtph.2015.10.024.

da Silva Filho, A.A., Resende, D.O., Fukui, M.J., Santos, F.F., Pauletti, P.M., Cunha, W.R., Silva, M.L.A., Gregório, L.E., Bastos, J.K., Nanayakkara, N.P.D., 2009. *In Vitro* antileishmanial, antiplasmodial and cytotoxic activities of phenolics and triterpenoids from *Baccharis dracunculifolia* DC. (asteraceae). Fitoterapia 80, 478–482. https://doi.org/10.1016/j.fitote.2009.06.007.

Dutra, R.C., Campos, M.M., Santos, A.R.S., Calixto, J.B., 2016. Medicinal plants in Brazil: pharmacological studies, drug discovery, challenges and perspectives. Pharmacol. Res. 112, 4–29. https://doi.org/10.1016/j.phrs.2016.01.021.

Finger, D., Machado, C.S., Torres, Y.R., Quináia, S.P., Thomaz, A.C.G., Gobbo, A.R., Monteiro, M.C., Ferreira, A.G., Sawaya, A.C.H.F., Eberlin, M.N., 2013. Antifungal bioassay-guided fractionation of an oil extract of propolis. J. Food Qual. 36, 291–301. https://doi.org/10.1111/jfq.12039.

Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro. http://floradobrasil.jbrj.gov.br/. (Accessed 5 March 2024).

Florão, A., Budel, J.M., Duarte, M., do, R., Marcondes, A., Rodrigues, R.A.F., Rodrigues, M.V.N., Santos, C.A., de, M., Weffort-Santos, A.M., 2012. Essential oils from *baccharis* species (asteraceae) have anti-inflammatory effects for human cells. J. Essent. Oil Res. 24, 561–570. https://doi.org/10.1080/10412905.2012.728081.

Fernandes, G.W., Oki, Y., Barbosa, M., 2021. *Baccharis* from Evolutionary and Ecological Aspects to Social Uses and Medicinal Applications. Springer Nature, Cham, Switzerland. https://doi.org/10.1007/978-3-030-83511-8.

Frizzo, C.D., Atti-Serafini, L., Laguna, S.E., Cassel, E., Lorenzo, D., Dellacassa, E., 2008. Essential oil variability in *baccharis Uncinella* DC. And *Baccharis dracunculifolia* DC. Growing wild in southern Brazil, Bolivia and Uruguay. Flavour Fragrance J. 23, 99–106. https://doi.org/10.1002/ffj.1862.

Gasparotto Junior, A., Boffo, M.A., Lourenço, E.L., Stefanello, M.E.A., Kassuya, C.A.L., Marques, M.A.C., 2009. Natriuretic and diuretic effects of *Tropaeolum majus* (tropaeolaceae) in rats. J. Ethnopharmacol. 122, 517–522. https://doi.org/10.1016/j.jep.2009.01.021.

Gasparotto Junior, A., Gasparotto, F.M., Lourenço, E.L.B., Crestani, S., Stefanello, M.E.A., Salvador, M.J., Silva-Santos, J.E., Marques, M.C.A., Kassuya, C.A.L., 2011. Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.: evidence for the inhibition of angiotensin converting enzyme. J. Ethnopharmacol. 134, 363–372. https://doi.org/10.1016/j.jep.2010.12.026.

Klider, L.M., Marques, A.A.M., Moreno, K.G.T., da Silva, G.P., Mizuno, G.A., Farias, K.D. S., Monteiro, L.M., de Almeida, V.P., Monchak, I.T., da Silva, D.B., Manfron, J., Gasparotto, Junior A., 2024. Pharmacological mechanisms involved in the diuretic activity of the ethanol-soluble fraction of *Baccharis milleflora* (less.) DC.-An ethnopharmacological investigation. J. Ethnopharmacol. 335, 118629. https://doi.org/10.1016/j.jep.2024.118629.

Loayza, I., Abujder, D., Aranda, R., Jakupovic, J., Collin, G.U.Y., Deslauriers, H., Jean, F. I., 1995. Essential oils *of Baccharis salicifolia, B. latifolia and B. dracunculifolia.* Phytochemistry 38, 381–389. https://doi.org/10.1016/0031-9422(94)00628-7.

Malone, M.H., Robichaud, R.C., 1962. A hippocratic screen for pure or crude drug materials. J. Nat. Prod. Lloydia 25, 320–331.

Mariano, L.N.B., Boeing, T., Silva, R.C.V., Silva, L.M., Gasparotto-Junior, A., Cechinel-Filho, V., Souza, P., 2022. Exotic medicinal plants used in Brazil with diuretic properties: a review. Chem. Biodivers. 19, e202200258. https://doi.org/10.1002/cbdv.202200258.

Mariano, L.N.B., Boeing, T., Cechinel-Filho, V., Niero, R., Silva, L.M., Souza, P., 2020. The acute diuretic effects with low-doses of natural prenylated xanthones in rats. Eur. J. Pharmacol. 884, 173432. https://doi.org/10.1016/j.ejphar.2020.173432.

Mc Lafferty, F.W., 2000. Wiley Registry of Mass Spectral Data with NIST Spectral Data, seventh ed. Database. John Wiley & Sons, Hoboken, USA [CD-ROM].

Mendes, T.C., Silva, G.R., Silva, A., Schaedler, M.I., Guarnier, L.P., Palozi, R.A.C., Signor, C.T., Dal Bosco, J.D., Auth, P.A., Amaral, E.C., Froelich, D.L., Soares, A.A., Lovato, E.C.W., Ribeiro-Paes, J.T., Gasparotto Junior, A., Lívero, F.A.R., 2021. Hepato- and cardioprotective effects of *Baccharis trimera* (less.) DC. Against multiple risk factors for chronic noncommunicable diseases. Biomed. Sci. 93, 1–16. https://doi.org/10.1590/0001-3765202120200899.

Mendonça, L.A.B.M., Matias, R., Zanella, D.F.P., Porto, K.R.A., Guilhermino, J.F., Moreira, D.L., Roel, A.R., Pott, A., Carvalho, C.M.E., 2020. Toxicity and phytochemistry of eight species used in the traditional medicine of sul-mato-grossense, Brazil. Braz. J. Biol. 80, 574–581. https://doi.org/10.1590/1519-6984.216406.

Minteguiaga, M., González, A., Cassel, E., Umpierrez, N., Fariña, L., Dellacassa, E., 2018. Volatile constituents from *baccharis* spp. L. (asteraceae): Chemical support for the conservation of threatened species in Uruguay. Chem. Biodivers. 15, e1800017. https://doi.org/10.1002/cbdv.201800017.

Minteguiaga, M., González, H.A., Ferreira, F., Dellacassa, E., 2021a. *Baccharis dracunculifolia* DC. In: Máthé, Á., Bandoni, A. (Eds.), Medicinal and Aromatic Plants of South America. Springer Nature, Cham, Switzerland, pp. 85–105. https://doi.org/10.1007/978-3-030-62818-5_5.

Minteguiaga, M., González, A., Catalán, C.A.N., Dellacassa, E., 2021b. Relationship between *Baccharis dracunculifolia* DC. And *B. microdonta* DC. (asteraceae) by their different seasonal volatile expression. Chem. Biodivers. 18, e2100064. https://doi.org/10.1002/cbdv.202100064.

Mishima, S., Yoshida, C., Akino, S., Sakamoto, T., 2005. Antihypertensive effects of Brazilian propolis: identification of caffeoylquinic acids as constituents involved in the hypotension in spontaneously hypertensive rats. Biol. Pharm. Bull. 28, 1909–1914. https://doi.org/10.1248/bpb.28.1909.

Mondello, L., 2008. Flavors and Fragrances of Natural and Synthetic Compounds/Mass Spectral Library. John Wiley & Sons, Hoboken, USA [CD-ROM], Version 1.3.

OECD, 2022. Organization for economic cooperation and development. Guidelines for testing of chemical. Acute oral toxicity: Up-and-Down Procedure (UDP), test No. 425, 1–29. Paris.

Oliveira, T.A., Michel, R.G., Snak, A.L., Pereira, R.A., Kagimura, F.Y., Cunha, M.A.A., Malfatti, R.M., 2014. *Baccharis dracunculifolia* with high levels of phenol compounds reduce blood glucose in healthy human. Afr. J. Pharm. Pharmacol. 8, 670–673. https://doi.org/10.5897/AJPP2013.3819.

Oliveira-Lima, J., Pereira, B.F., Valim, J.R.T., Gazoni, T., 2019. Effects of ingested *Baccharis dracunculifolia* D.C. (asteraceae) extract in the liver of *Prochilodus lineatus* fish. Microsc. Res. 7, 27–38. https://doi.org/10.4236/mr.2019.73003.

Oparil, S., Acelajado, M.C., Bakris, G.L., Berlowitz, D.R., Cífková, R., Dominiczak, A.F., Grassi, G., Jordan, J., Poulter, N.R., Rodgers, A., Whelton, P.K., 2018. Hypertension. Nat. Rev. Dis. Primers 4, 18014. https://doi.org/10.1038/nrdp.2018.14.

Pan, S.H.U., Jun-Li, H.O.N.G., Gang, W.U., Bo-Yang, Y.U., Min-Jian, Q.I.N., 2010. Analysis of flavonoids and phenolic acids in *iris Tectorum* by HPLC-DAD-ESI-MS^n. Chin. J. Nat. Med. 8, 202–207. https://doi.org/10.3724/SP.J.1009.2010.00202.

Rafi, M., Hayati, F., Umar, A.H., Septaningsih, D.A., Rachmatiah, T., 2023. LC-HRMS-based metabolomics to evaluate the phytochemical profile and antioxidant capacity of *Cosmos caudatus* with different extraction methods and solvents. Arab. J. Chem. 16, 105065. https://doi.org/10.1016/j.arabjc.2023.105065.

Resende, F.A., Alves, J.M., Munari, C.C., Senedese, J.M., Sousa, J.P.B., Bastos, J.K., Tavares, D.C., 2007. Inhibition of doxorubicin-induced mutagenicity by *Baccharis dracunculifolia*. Mutat. Res. 634, 112–118. https://doi.org/10.1016/j.mrgentox.2007.06.008.

Roberto, M.M., Matsumoto, S.T., Jamal, C.M., Malaspina, O., Marin-Morales, M.A., 2016. Evaluation of the genotoxicity/mutagenicity and antigenotoxicity/antimutagenicity induced by propolis and *Baccharis dracunculifolia*, by *In Vitro* study with HTC cells. Toxicol. Vitro 33, 9–15. https://doi.org/10.1016/j.tiv.2016.02.005.

Rodrigues, D.M., De Souza, M.C., Arruda, C., Pereira, R.A.S., Bastos, J.K., 2020. The role of *Baccharis dracunculifolia* and its chemical profile on green propolis production by *apis Mellifera*. J. Chem. Ecol. 46, 150–162. https://doi.org/10.1007/s10886-019-01141-w.

Roush, G.C., Kaur, R., Ernst, M.E., 2014. Diuretics: a review and update. Cardiovasc. Pharmacol. Core Rev. 19, 5–13. https://doi.org/10.1177/1074248413497257.

Sellers, R.S., Mortan, D., Michael, B., Roome, N., Johnson, J.K., Yano, B.L., Perry, R., Schafer, K., 2007. Society of toxicologic pathology position paper: organ weight recommendations for toxicology studies. Toxicol. Pathol. 35, 751–755. https://doi.org/10.1080/01926230701595300.

Souza, P., Mariano, L.N.B., Cechinel-Zanchett, C.C., Cechinel-Filho, V., 2021. Promising medicinal plants with diuretic potential used in Brazil: state of the art, challenges, and prospects. Planta Med. 87, 24–37. https://doi.org/10.1055/a-1257-0887.

Souza, M.M.Q., Silva, G.R., Cola, I.M., Siva, A.O., Schaedler, M.I., Guarnier, L.P., Palozi, R.A.C., Barboza, L.N., Menetrier, J.V., Froelich, D.L., Auth, P.A., Veiga, A.A., Souza, L.M., Lovato, E.C.W., Ribeiro-Paes, J.T., Gasparotto Junior, A., Lívero, F.A.R., 2020. *Baccharis trimera* (less.) DC: an innovative cardioprotective herbal medicine against multiple risk factors for cardiovascular disease. J. Med. Food 23, 1–9. https://doi.org/10.1089/jmf.2019.0165.

Tolouei, S.E.L., Palozi, R.A.C., Tirloni, C.A.S., Marques, A.A.M., Schaedler, M.I., Guarnier, L.P., Silva, A.O., Almeida, V.P., Budel, J.M., Souza, R.I.C., Santos, A.C., Silva, D.B., Lourença, E.L.B., Dalsenter, P.R., Gasparottto Junior, A., 2019. Ethnopharmacological approaches to *Talinum paniculatum* (jacq.) gaertn. - exploring cardiorenal effects from the Brazilian cerrado. J. Ethnopharmacol. 238, 111873. https://doi.org/10.1016/j.jep.2019.111873.

Tomazzoli, M.M., do Amaral, W., Cipriano, R.R., de Cássia, Tomasi, Gomes, E.N., Gomes, E.N., Ferriani, A.P., de Noronha Sales Maia, B.H.L., Deschamps, C., 2021. Chemical composition and antioxidant activity of essential oils from populations of *Baccharis dracunculifolia* DC. In southern Brazil. Braz. Arch. Biol. Technol. 64, 1–14. https://doi.org/10.1590/1678-4324-2021190253.

Vargas, F., Romecín, P., García-Guillén, A.I., Wangesteen, R., Vargas-Tendero, P., Paredes, M.D., Atucha, N.M., García-Estañ, J., 2018. Flavonoids in kidney health and disease. Front. Physiol. 9, 1–12. https://doi.org/10.3389/fphys.2018.00394.

Veiga, R.D.S., Marcucci, M.C., Queiroga, C.L., Sawaya, A.C.H.F., da Silva, C.B., Sasagawa, S.M., Navarini, A., Marchetti, G., Marinho, G.T., Fukunaga, E.T., Gonçalves, C.P., Mimica, M.J., 2023. Synergistic effect of *Baccharis dracunculifolia* DC and green propolis with antimicrobial drugs against Staphylococcus aureus. Braz. J. Nat. Sci. 5, e1782023. https://doi.org/10.31415/bjns.v5i1.178.

Wei, J., Zhao, M., Meng, K., Xia, G., Pan, Y., Li, C., Zhang, W., 2022. The diuretic effects of coconut water by suppressing aquaporin and renin–angiotensin–aldosterone system in saline-loaded rats. Front. Nutr. 9, 1–10. https://doi.org/10.3389/fnut.2022.930506.

Wiley/NIST, 2023. Wiley Registry/NIST Mass Spectral Library 2023. [CD-ROM]. John Wiley & Sons, Hoboken, USA.