

Secondary Metabolites, Photosynthetic Pigments, Mineral Composition, and Antiproliferative Screening of Hydrodistillation Wastewater from Two *Campomanesia* (Myrtaceae) Species

Metabólitos Secundários, Pigmentos Fotossintetizantes, Composição Mineral e Triagem Antiproliferativa do Resíduo Aquoso da Destilação de Duas Espécies de *Campomanesia* (Myrtaceae)

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Hydrodistillation wastewater generated during essential oil extraction is commonly discarded, even though it may contain significant amounts of bioactive compounds. In this study, the chemical composition and antiproliferative screening of wastewater obtained from *Campomanesia adamantium* (Cambess.) O.Berg (CAWW) and *Campomanesia sessiliflora* (O.Berg) Mattos (CSWW) leaves were evaluated. Total phenolic compounds, flavonoids, tannins, anthocyanins, photosynthetic pigments, mineral composition, and infrared spectral profiles were determined. Both samples exhibited expressive levels of phenolic compounds, with CAWW presenting higher total phenolic and flavonoid contents, while CSWW showed higher tannin levels and detectable anthocyanins. Carotenoids were detected in both samples, and chlorophyll α was identified only in CSWW. Mineral analysis revealed the presence of macro and micronutrients in both wastewaters. In the antiproliferative screening, CAWW and CSWW inhibited the growth of selected tumor cell lines (HT-29, MCF-7, and UACC-62) at less than 250 $\mu\text{g mL}^{-1}$. The results indicate that hydrodistillation wastewater from *Campomanesia* species retains chemically relevant constituents and demonstrates biological potential. This evidence advances the chemical characterization of the genus and highlight the value of this by-product as a promising resource for future research and applications.

Keywords: Molecular absorption; infrared spectroscopy; aromaticity index; guavira.

1. Introduction

Plant metabolites can be divided into two groups: primary and secondary metabolites. Primary metabolites are directly involved in plant growth, development, and cellular homeostasis, whereas secondary metabolites are not essential for basic metabolic processes but play important roles in plant defense and adaptation to biotic and abiotic environmental stresses.¹ In this way, different parts of the plant produce distinct metabolites depending on developmental and environmental needs.²

Secondary metabolites of plants exhibit a wide diversity of structures and can exhibit different biological activities.³ For this reason, some plants, called medicinal plants, have been used for healing since the beginning of humanity.⁴ This popular use of medicinal plants occurs in various forms, such

as teas, hip baths, ointments, and tinctures.⁵

This context involves *Campomanesia sessiliflora* (O.Berg) Mattos and *Campomanesia adamantium* (Cambess.) O. Berg, species traditionally employed in folk medicine. Native to the Brazilian Cerrado, these fruit-bearing plants are valued not only for their edible fruits but also for leaf infusions, which are commonly used in traditional practices to treat gastrointestinal disorders, particularly diarrhea.^{6,7} Some medicinal plants are considered aromatic, producing a mixture of volatile organic compounds characterized by the presence of monoterpenes, sesquiterpenes, diterpenes, low-molecular-weight hydrocarbons, their oxygenated derivatives, and phenylpropanoids, namely, as essential oils.⁸ Essential oils typically exhibit higher bioactivity and are studied for medicinal and cosmetic applications.⁹

However, the current hydrodistillation process used to obtain essential oils produces substantial waste that hasn't been explored. In addition, essential oils normally have low yield, rarely higher than 5%, increasing the waste production to obtain a low amount of product.¹⁰

The essential oils of *C. sessiliflora* leaves have been investigated for their antimicrobial properties,¹¹ while studies on the essential oil of *C. adamantium* leaves have demonstrated antimicrobial,^{11–13} antioxidant,¹³ anti-inflammatory¹⁴ activities, as well as repellent and insecticide effects against *Aedes aegypti* and *Culex quinquefasciatus*.¹⁵ However, reported yields of *C. sessiliflora* leaves essential oil in the literature range from 0.19%¹¹ to 2.5%,¹⁴ which limits its feasibility for industrial applications. Valuing wastewater from the hydrodistillation process could be an eco-friendly way to make the industrial production of essential oils from these species a reality.

There are various classes of secondary metabolites with biological activity, among which phenolic compounds are particularly prominent.¹⁶ These compounds are generally characterized by the presence of one or more phenolic hydroxyl groups in their chemical structures.¹⁷ In this context, certain subclasses—such as flavonoids, tannins, and anthocyanins—have been extensively investigated for their antiproliferative properties.¹⁸ In addition, plant matrices can serve as sources of inorganic elements that are also relevant to human health.¹⁹

Among the phenolic compounds, the flavonoids stand out as a major class, exhibiting a C6–C3–C6 structure composed of two aromatic rings linked by an oxygenated heterocycle.²⁰ Their biological activity is directly influenced by the number and position of hydroxyl and methoxy groups,²¹ with hydroxylation in the B ring enhancing antioxidant capacity through electron donation and stabilization of free radicals.²²

In this context, aqueous extracts from *C. adamantium* have been consistently reported as sources of phenolic acids and flavonoids, with gallic acid and ellagic acid as major constituents, alongside rutin and quercetin.^{23,24} Similarly, *C. sessiliflora* has been shown to contain relevant phenolic compounds, including gallic and syringic acids.^{25,26}

The present study aimed to evaluate the content of the secondary metabolites, photosynthetic pigments, mineral composition, and antiproliferative screening of hydrodistillation wastewater from *C. adamantium* and *C. sessiliflora*.

2. Experimental

2.1. Reagent and equipment

Distilled water and Type I ultrapure water (≥ 18 M Ω , Milli-Q system, Millipore, USA) were used. Hydrogen

peroxide, ethanol, aluminum chloride, sodium carbonate, and potassium chloride were purchased from Êxodo Científica (Brazil). Methanol, nitric acid, and sodium acetate were obtained from Vetec Química Fina (Brazil). Rutin, gallic acid, and tannic acid standards; multi-element standard solutions (Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, S, Zn); Folin–Ciocalteu and Folin–Denis reagents; doxorubicin; gentamicin sulfate; trichloroacetic acid; sulforhodamine B; and acetic acid were acquired from Sigma-Aldrich (USA). RPMI 1640 culture medium and fetal bovine serum (FBS) were purchased from Gibco (USA).

A Clevenger-type apparatus was used for hydrodistillation. Freeze-drying was performed using an Alpha 1–2 LD Plus freeze dryer (Martin Christ Gefriertrocknungsanlagen, Germany). UV–Vis analyses were carried out using a spectrophotometer (UV-M51, BEL Photonics, Brazil). Infrared analyses were performed on a spectrophotometer (Thermo Nicolet Nexus 670, USA) coupled to a photoacoustic detector (MTEC-300, MTEC Photoacoustics, USA). Mineral determination was performed using an inductively coupled plasma optical emission spectrometer (ICP-OES, ICPE-9000, Shimadzu Corporation, Japan). Additional equipment included a digester block (TE007-A, TECNAL, Brazil), a CO₂ incubator (BJPX-C160II, BIOBASE, China), an ultrasonic bath (Q335D, Quimis, Brazil), and an analytical balance (MX204/A, Mettler-Toledo, Brazil).

2.2. Collection and recording of the plants

The collection of *C. sessiliflora* and *C. adamantium* leaves was in Dourados, Mato Grosso do Sul State, Brazil. The herbarium specimens were identified and deposited in the Federal University of Grande Dourados herbarium at code 2133 for *C. sessiliflora* and 2192 for *C. adamantium*.

2.3. Obtain the wastewater from hydrodistillation

The hydrodistillation was performed in a Clevenger-like extractor, using 240 g of fresh leaves in 3 L of distilled water, yielding a concentration of 80 g L⁻¹ of fresh leaves. The extraction was performed for 4 h for both species. After the extraction of essential oil, the leaves were separated from the wastewater by filtration. The wastewater was freeze-dried at a vacuum of 0.045 mbr and a temperature of -42 °C (Alpha 1-2LD Plus, Christ).

2.4. Screening of the absorption ultraviolet (UV), visible (Vis), and infrared (IR) regions

The UV/Vis screening was performed on a spectrophotometer using the MetaSpace Pro program. The

freeze-dried wastewater samples were prepared at 1 mg mL⁻¹ and analyzed in a quartz cuvette between 190 and 800 nm with 1 nm resolution.

The spectrophotometer with a photoacoustic detector was used to analyze the freeze-dried extracts in the infrared region. To avoid interference, a purge was performed with dry air in the spectrophotometer and, between readings, with helium in the photoacoustic chamber. A black reference was used for equipment calibration. The reading was performed between 4000 and 400 cm⁻¹, with 128 scans per sample and a resolution of 8 cm⁻¹. The analysis was performed on the wastewater dry extract. The aromatic index was calculated by Equation 1, described by Amaral *et al.*²⁶

$$\text{Aromatic index} = \frac{\text{Absorption intensity}_{1620 \text{ cm}^{-1}}}{\text{Absorption intensity}_{2920 \text{ cm}^{-1}}} \quad (1)$$

2.5. Quantification of total secondary metabolites

The quantification of the secondary metabolites was performed on a freeze-dried extract prepared at 1 mg mL⁻¹ in distilled water. All analyses were performed in triplicate, using a glass cuvette and distilled water as a blank.

2.5.1. Flavonoids content

The reaction was performed using 1 mL of samples for 1 mL of aluminum chlorate 0.15 mol L⁻¹ in methanol for 15 min.²⁷ The reaction was monitored at 430 nm, using a standard curve of rutin for quantification, with a linear range of 0.1-100 µg mL⁻¹ (a = 0.0019; b = 0.0105; R² = 0.9990). The result was expressed in mg of rutin equivalent (RE) per g of freeze-dried extract.

2.5.2. Phenolic compounds content

The colorimetric method of Folin-Ciocalteu was used.²⁷ To do this, 0.5 mL of Folin-Ciocalteu reagent (1:10 v/v) and 1 mL of distilled water were added to 0.1 mL of the sample.¹⁶ After 1 min, 1.5 mL of sodium carbonate (1.89 mol L⁻¹) was added, and the mixture was left in the dark for 2 h. The reaction was monitored at 760 nm, using a standard curve of gallic acid for quantification, with a linear range of 10-1000 µg mL⁻¹ (a = 0.0008; b = 0.0015; R² = 0.9875). The result was expressed in mg of gallic acid equivalent (GAE) per g of freeze-dried extract.

2.5.3. Tannins content

The total tannin content was determined using the Folin-Denis method²⁸ with adjustments to the volumes, while maintaining the proportions, as described by de Castro *et al.*²⁹ For each 500 µL of sample, 500 µL of Folin-Denis reagent was added, followed by 500 µL of sodium carbonate (0.75 mol L⁻¹), and allowed to react for 2 h.

The reaction was monitored at 725 nm, using a standard curve of tannic acid for quantification, with a linear range of 0.5-80 µg mL⁻¹ (a = 0.03837; b = 0.009; R² = 0.99826). The result was expressed in mg of tannic acid equivalent (TAE) per g of freeze-dried extract.

2.5.4. Anthocyanins

The quantification of anthocyanins was performed using the pH differential method.³⁰ Two separate dilutions were prepared: 1 mL of extract in 25 mL of potassium chloride buffer (0.025 mol L⁻¹; pH 4.5) and 1 mL of extract in 25 mL of sodium acetate buffer (0.4 mol L⁻¹; pH 1). The samples were kept protected from light for 30 min, after which the absorbance was measured at 520 nm and 700 nm for both buffered solutions. The content was calculated using Equation 2.

$$A = \frac{[(\lambda_{520\text{nm}} - \lambda_{700\text{nm}})_{\text{pH } 1.0} - (\lambda_{520\text{nm}} - \lambda_{700\text{nm}})_{\text{pH } 4.5}] \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l} \quad (2)$$

where A is the anthocyanin content, MW is the molecular weight of the reference, DF is the dilution factor, ϵ is the molar extinction coefficient, and l is the path length of the cuvette. The calculations used cyanidin-3-glucoside as the reference (MW = 449.2 g mol⁻¹; ϵ = 26900 L mol⁻¹ cm⁻¹).

2.6. Content of photosynthetic pigments

The freeze-dried extract was solubilized in ethanol at 1 mg mL⁻¹, then filtered through an analytical filter to remove insoluble particulate material. The absorbance was then measured using a spectrophotometer at 655, 649, and 470 nm, subtracting the ethanol absorbance at each wavelength. Chlorophyll α was calculated using Equation 3, chlorophyll β using Equation 4, and carotenoids using Equation 5, as described by Lichtenthaler and Wellburn.³¹ The experiment was performed in triplicate.

$$\text{chlorophyll } \alpha = (13.95 \times \text{Abs}_{665}) - (6.88 \times \text{Abs}_{649}) \quad (3)$$

$$\text{chlorophyll } \beta = (24.96 \times \text{Abs}_{649}) - (7.32 \times \text{Abs}_{665}) \quad (4)$$

$$\text{Carotenoids} = \frac{1000 \times \text{Abs}_{470\text{nm}} - (2.05 \times \text{chlorophyll } \alpha) - (114.8 \times \text{chlorophyll } \beta)}{245} \quad (5)$$

2.7. Mineral contents

Type I ultra-purified water (≥ 18 M Ω) from the Milli-Q system (Millipore, USA) was employed for sample preparation. Glassware and bottles designated for storing and preparing samples were decontaminated with a 10% nitric acid solution (v:v) before use. They were soaked for 24 h and washed thoroughly with ultra-purified water.

For each extraction, 500 mg of freeze-dried sample was infused into borosilicate glass tubes within a digester

block. The samples underwent pre-digestion with 3.5 mL of nitric acid for 24h at room temperature (25 °C), followed by digestion for 45 min at 150 °C. During this process, 1.5 mL of hydrogen peroxide was added, and the samples were maintained in the digester block for an additional 45 min at the same temperature. A blank solution was prepared, containing only the nitric acid and hydrogen peroxide mixture. The resulting solutions were prepared for analysis in ultrapure water and left to cool.

The analysis used inductively coupled plasma optical emission spectroscopy in the spectral range of 167-800 nm. The equipment was calibrated using solutions ranging from 10-5,000 µg L⁻¹, containing elements such as Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, S, and Zn.

Operating parameters were set as follows: Radiofrequency power 1500 W, Radiofrequency generator 27.12 MHz, Sample aspiration flow rate 1.5 mL min⁻¹, Argon flow rate (L min⁻¹) – 15.00 (plasma), 1.0 (auxiliary), and 0.45 (nebulizer). The result was expressed in mg per kg⁻¹ of freeze-dried extract. The experiment was performed in triplicate.

2.8. Antiproliferative assay

The *in vitro* antiproliferative activity was evaluated using doxorubicin as a positive control, as described by Monks *et al.*³² For the activity test, 100 µL of cells were plated in RPMI/SFB/gentamicin medium in 96-well plates. The plates were incubated for 24h at 37 °C in a 5% carbon dioxide atmosphere. After this period, a control plate was fixed by adding trichloroacetic acid to determine the number of cells at the time of drug addition. The freeze-dried extracts were evaluated at concentrations ranging from 0.0625 to 250 µg mL⁻¹ in distilled water and incubated for 48 h. After the incubation period, the plates were stained by adding 50 µL of 0.4% sulforhodamine B protein dye dissolved in 1% acetic acid and incubating at 4 °C for 30 min. The dye bound to cellular proteins was solubilized using a Trizma Base solution at 10 µM and pH 10.5 for 5 min under ultrasound. The result was obtained in absorbance at 560 nm. The TGI (Total Growth Inhibition) was determined in triplicate in independent experiments. Values were determined by non-linear (sigmoidal) regression analysis using the Origin 7.5 program ($r^2 > 0.9$).

2.9. Statistical analysis

The statistical analysis of the data was performed using R Studio software.³³ Initially, the homogeneity of variance and normality were analyzed using the Shapiro-Wilk test. For analyses comparing two samples, the t-test was used with a significance level of 5%.

3. Results and Discussion

Initially, molecular absorption in the ultraviolet and visible regions was measured for the samples (Table 1). The CSWW and CAWW showed absorption bands at 250, 300, and 350 nm. These regions may be associated with the presence of phenolic compounds, as phenols absorb in the ultraviolet region due to aromatic rings with conjugated π -electron systems. These structures allow $\pi \rightarrow \pi^*$ electronic transitions when exposed to UV radiation. The presence of hydroxyl and other substituent groups can extend conjugation and modify electron density, often shifting absorption to longer wavelengths and increasing intensity.³⁴⁻³⁸

The CSWW and CAWW have similar UV absorption profiles, with absorption bands at 257, 306, and 352 nm, but differ in intensity at 352 nm (Figure 1). This absorption band at 352 nm is not observed in the spectra reported for the infusion and decoction of *C. sessiliflora* leaves,³⁴ nor in those of *C. adamantium* leaves³⁵ or the infusion of *C. guazumifolia* leaves.³⁶

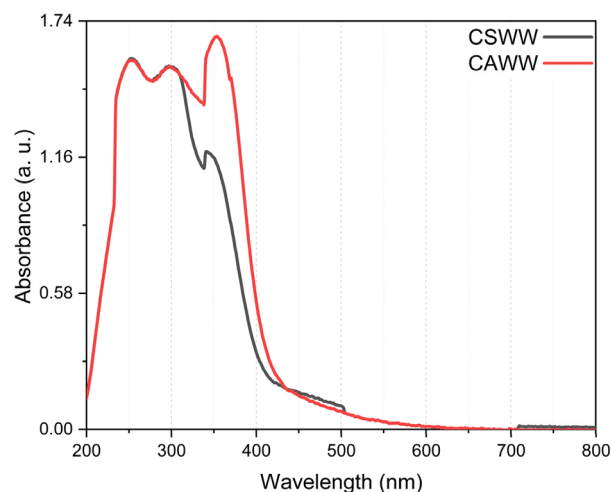


Figure 1. Molecular absorption spectrum of the CAWW and CSWW

An increase in the intensity of the 352 nm band can be observed in the leaf extracts of *C. adamantium* in the study by de Castro *et al.*,³⁷ as the proportion of ethanol in the extraction rises, thereby favoring the recovery of the chalcone 5,7-dihydroxy-6-methylflavanone. Consequently, the presence of the absorption band at 352 nm in the samples may be associated with the presence of chalcones, given that Rammohan *et al.*³⁸ reported this class of compounds to exhibit two characteristic absorption bands: the first between 200 and 270 nm, and the second between 340 and 390 nm.

Moreover, chalcones have been reported in different extracts of plants from this genus: hydroethanolic extract of the fruit peels and leaves of *C. adamantium*,^{37,39}

dichloromethane and ethanolic extracts of the fruits, methanolic, hexanic, and ethyl acetate extracts of the leaves of *C. adamantium*,^{20,40-41} ethanolic extracts of the fruits and leaves of *C. pubescens*,⁴²⁻⁴³ and hydroethanolic and ethanolic extracts of the leaves of *C. xanthocarpa*.⁴⁴

Bands close to 257 nm have been observed in the infusion and decoction of *C. sessiliflora* leaves,³⁴ in the infusion of *C. guazumifolia* leaves,³⁶ and in the ethanolic, aqueous, and hydroethanolic extracts of *C. adamantium* leaves.³⁷ Meanwhile, bands close to 306 nm were observed in the ethanolic extracts of the fruit peels of *C. sessiliflora* and *C. guazumifolia*²⁹ and in the infusion and decoction of *C. adamantium* leaves.³⁵ The presence of absorption bands in the UV region in extracts of plants from the genus *Campomanesia* has been attributed to the presence of phenolic compounds.^{29,34,35-37}

The wastewater extracts also exhibited similar profiles in the molecular absorption spectra in the infrared fingerprint region (1800 to 700 cm⁻¹), with differences in the intensity of the absorption bands (Figure 2).

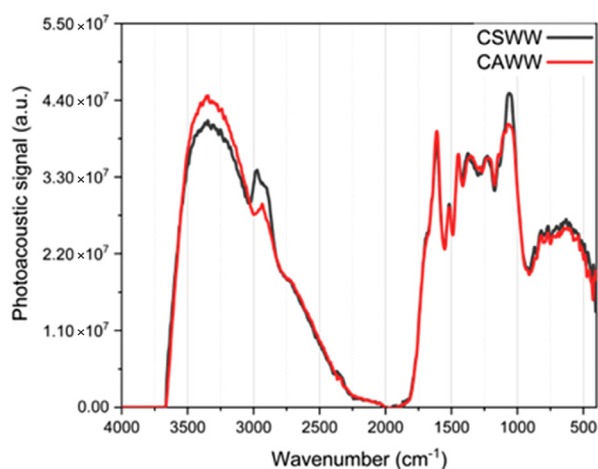


Figure 2. Normalized mean of the spectrum in the infrared region with Fourier transform and photoacoustic detector of CAWW and CSWW

The samples exhibit bands that may be associated with hydroxyl vibrations (3347, 3340, 1450, 1376, 1230, and 1230 cm⁻¹), conjugated double bonds (1515 cm⁻¹), and C-O bonds (1376, 1349, 1145, and 1141 cm⁻¹), which may occur in phenolic compounds.^{26,45-49} Some bands may be associated with the presence of nitrogen-containing compounds in the samples (2977, 1612, 1608, 1515, and 1376 cm⁻¹).^{26,46-48,40} Furthermore, bands associated with ester vibrations may result from the presence of free sugars or those linked to phenolic compounds (1612, 1608, 1376, 1349, 1145, 1141, and 1052 cm⁻¹)^{26,45-49} (Table 1).

Spectra with similar bands have been reported for the wastewaters in the ethanolic, aqueous, and hydroethanolic extracts of *C. adamantium* leaves³⁷ and in the infusion of *C. guazumifolia* leaves,³⁶ although with distinct intensities.

The aromatic index was initially proposed to estimate the presence of phenolic compounds in humic compounds.⁵¹ However, Amaral *et al.*²⁶ reported a correlation between the aromatic index and the total phenolic content of hydroethanolic extracts of *Psidium guajava* (Linn.) (Myrtaceae). The wastewater of *C. adamantium* exhibited an aromatic index of 1.376, which is higher than that of *C. sessiliflora*, which was 1.155, indicating a greater phenolic content in the wastewater of *C. adamantium*. Amaral *et al.*²⁶ reported aromatic indices of 1.432 for the extract obtained with 50% ethanol and 1.225 for the extract obtained with 70% ethanol, values similar to those obtained for the wastewaters.

CAWW exhibited higher levels of total phenolic compounds and flavonoids compared to CSWW, but had a lower tannin content and no detectable anthocyanins in the freeze-dried extract (Table 2). In agreement with the spectral behavior previously discussed, the higher phenolic compounds and flavonoid contents in CAWW are consistent with the greater absorption intensity observed around 350 nm in the UV-Vis spectrum.

Table 1. Photoacoustic signal peaks by infrared spectroscopy and associated functional groups of CSWW and CAWW

CSWW (cm ⁻¹)	CAWW (cm ⁻¹)	Associated Functional Group	Reference
3347	3340	-OH	34, 38
2977	—	-N-H, -CH ₂ or CH ₃	36, 39
—	2935	-CH ₂ or CH ₃	34-35, 38
1612	1608	-N-H, -C=C, -C=O or C-C-O	15, 35-37
1515	1515	-C=C or -N-H	15, 35-36
1450	1450	-C-O or -C-O-H	15
1376	—	C-O, C-C, C-C-O or CH ₂	35, 36
—	1349	C-C-O or -C-H (Aromatic)	15, 38
1230	1222	-O-H	36-37
1145	1141	-C-O	36
1052	1052	-C-OH, -C-O-C or -C-C-R	34, 38

Table 2. Secondary metabolites content in CSWW and CAWW

Class	CSWW	CAWW
Phenolic compounds (mg GAE g ⁻¹ ± SD)	298.13 ± 3.00 ^b	313.80 ± 6.45 ^a
Flavonoids (mg RE g ⁻¹ ± SD)	48.86 ± 0.77 ^b	71.00 ± 1.66 ^a
Tannins (mg TAE g ⁻¹ ± SD)	51.85 ± 2.00 ^a	38.51 ± 0.72 ^b
Anthocyanins (mg cyd-3-glu g ⁻¹ ± SD)	1.30 ± 0.01 ^a	-

SD = Standard deviation; GAE = Gallic acid equivalent; RE = Rutin equivalent; TAE = Tannic acid equivalent; cyd-3-glu = Cyanidin-3-glucoside equivalent. Different letters in the row between species indicate a significant difference ($p < 0.05$) in the t-test. - = Not detected.

The anthocyanin content of CSWW is lower than that reported by de Castro *et al.*²⁹ for the ethanolic extracts of the fruit peels of *C. sessiliflora* and *C. guazumifolia* from Mato Grosso do Sul state, Brazil. Schmidt *et al.*⁵² identified two anthocyanins in the acidified methanolic extract of *C. xanthocarpa* fruit.

The content of phenolic compounds present in the wastewater (Table 2) are higher than those reported for ethanolic extracts of *C. adamantium* leaves from Paraná State, Brazil by Pascoal *et al.*,⁵³ which presented 35.04 mg GAE g⁻¹, and are also above the values obtained for *C. xanthocarpa* leaves extracted with 70% ethanol (99.19 mg GAE g⁻¹), collected in January in Mato Grosso do Sul, Brazil, as described by da Silva *et al.*⁴⁴ However, the phenolic levels detected in the wastewater are within the range reported for more concentrated ethanolic extracts of *Campomanesia* leaves from Mato Grosso do Sul, Brazil, such as those described by Catelan *et al.*,⁵⁴ which reached 477.99 mg GAE g⁻¹ for *C. adamantium* and 435.67 mg GAE g⁻¹ for *C. sessiliflora*. Similarly, Loureiro *et al.*⁵⁵ reported phenolic contents ranging from 201.40 to 356.40 mg GAE g⁻¹ in different hydroethanolic extracts of *C. sessiliflora* leaves from Mato Grosso do Sul, Brazil, demonstrating that the wastewater generated during hydrodistillation still retains expressive levels of phenolic compounds.

Regarding flavonoids, the results (Table 2) are comparable to those reported for aqueous extracts of *C. adamantium* fruits collected in Mato Grosso do Sul, Brazil (55.29–65.93 mg RE g⁻¹) by Carnevali *et al.*⁵⁶ and infusion and decoction extracts of *C. sessiliflora* leaves collected in Mato Grosso do Sul, Brazil, which ranged from 47.58 to 63.39 mg RE g⁻¹.³⁴ Although higher flavonoid contents have been described for ethanolic extracts of *Campomanesia* leaves, reaching values above 300 mg RE g⁻¹,⁵⁴ the concentrations observed in the wastewater reinforce the potential of this by-product as a source of flavonoid compounds.

In contrast, tannin levels in CSWW and CAWW (Table 2) are lower than those reported for ethanolic extracts of *C. guazumifolia* peels, which reached 68.18 mg TAE g⁻¹,²⁹ but are comparable to values found in leaf collected in

Mato Grosso do Sul, Brazil, infusions of the same species (62.29 mg TAE g⁻¹).³⁶ Tannins are polymerized molecules, so maybe the heat of the hydrodistillation process breaks down part of the tannin into smaller molecules, such as gallic acid and ellagic acid.⁵⁷

Overall, the results demonstrate that the hydrodistillation wastewater from *Campomanesia* leaves contains significant amounts of secondary metabolites after essential oil extraction. When compared with conventional extraction methods reported in the literature, the levels observed in wastewater are frequently comparable to those obtained with aqueous preparations such as infusions and decoctions, and in some cases approach values reported for hydroethanolic extracts. These findings highlight the chemical richness of this by-product and reinforce its potential for further valorization and application as a source of bioactive compounds.

Chlorophyll α , β , and carotenoids are pigments that play a role in the process of photosynthesis. The literature review by Ebrahimi *et al.*⁵⁸ reports that the primary commercial use of chlorophyll is as a coloring agent in the cosmetic and food industries. Additionally, González-Peña *et al.*⁵⁹ report that carotenoids provide important health benefits, as some of them possess provitamin A activity—that is, they can be converted into vitamin A in the human body—and are therefore widely used in dietary supplements.

Chlorophyll α was detected only in CSWW, with a concentration of 48.94 ± 6.11 ng g⁻¹, while it was not detected in CAWW. Chlorophyll β was not detected in either wastewater sample. Carotenoids were quantified in both samples, with CSWW presenting significantly higher levels (260.41 ± 4.75 ng g⁻¹) compared to CAWW (86.94 ± 3.67 ng g⁻¹ ($p < 0.05$)). These results indicate marked differences in the pigment profiles of the two species, particularly regarding carotenoid content, which was substantially higher in the wastewater derived from *C. sessiliflora*.

The aqueous residues showed low levels of chlorophyll α and β pigments when compared to the results obtained by de Castro *et al.*²⁹ for the ethanolic extract of the fruit peels of *C. sessiliflora* and *C. guazumifolia*; however, the levels of carotenoids were higher than those reported in the

literature for the species *C. sessiliflora*. The low levels of photosynthetic pigments are possibly associated with prolonged heating during the oil extraction process, as these molecules tend to degrade with heat.⁶⁰

Element composition is an important consideration, especially if the extracts from wastewater are intended for food use. The major elements in CSWW were Ca, K, Fe, and P, while CAWW contained Ca, Fe, K, and P (Table 3). These four elements are considered crucial for bone metabolism, with deleterious effects occurring when adequate amounts are not consumed.⁶¹

Table 3. Elemental composition of freeze-dried CSWW and CAWW

Element	CSWW (mg kg ⁻¹ ± SD)	CAWW (mg kg ⁻¹ ± SD)
Na	219.00 ± 11.72	263.00 ± 17.60
K	923.00 ± 11.59	871.00 ± 12.77
Ca	1278.00 ± 43.50	1000.00 ± 69.29
Mg	613.00 ± 8.89	591.00 ± 11.53
P	776.00 ± 9.29	758.00 ± 12.53
Fe	879.00 ± 8.14	829.00 ± 8.02
Al	90.00 ± 3.51	72.00 ± 4.04
Zn	6.50 ± 0.15	5.60 ± 0.35
Cu	13.00 ± 0.58	10.00 ± 0.57
Mn	48.00 ± 1.53	43.00 ± 1.00

SD = Standard deviation.

The increase in sodium consumption in foods has led to a rise in reports of heart disease; thus, the World Health Organization has determined a Na-to-K ratio of ≤ 1.0 as optimal and ≤ 2.0 as the suboptimal goal.⁶² Both wastewaters have excellent Na-to-K ratios, with values of 0.24 for CSWW and 0.30 for CAWW. Therefore, adding these extracts to food products will help achieve good Na-to-K ratios.

However, the dry extract of CSWW and CAWW cannot be considered rich source of minerals when compared with fruits of the *Campomanesia* genus.⁶³⁻⁶⁵ The freeze-dried wastewater extract (1 kg) contains less K than 1 kg of fresh fruit from *C. guazumifolia*,⁶³ *C. adamantium*,⁶⁴ and *C. xanthocarpa*,⁶⁵ but it has higher levels of Ca, Fe, P, Mg, Mn, Zn, and Cu. This highlights the great potential for incorporating freeze-dried wastewater extracts into food products. Cu, Mn, and Zn are required in smaller amounts in the human diet; they are considered essential for human health, as they serve as crucial components of various enzymes in the human body.²⁵

The samples did not affect the growth of VERO cells (monkey renal epithelium) (Table 4). Compared to isolated compounds, the extracts are considered inactive. Samples that show total growth inhibition at concentrations between 6.25–15 µg mL⁻¹ can be considered moderate, and above 50 µg mL⁻¹ inactive.⁶⁶ However, in this study, a screening for

potential antiproliferative activity (0.0625 to 250 µg mL⁻¹). Both CAWW and CSWW showed inhibition: colon (HT-29), breast (MCF-7), and melanoma (UACC-62), but with different inhibitory concentrations (Table 4). However, compared to literature values, the samples are considered inactive.

Table 4. Antiproliferative activity (GI₅₀, µg mL⁻¹) of freeze-dried wastewaters from *C. sessiliflora* wastewater (CSWW) and *C. adamantium* wastewater (CAWW) against human tumor and normal cell lines.

Cell Line	Type	CAWW	CSWW
Vero	Non-tumor	>250	>250
PC-3	Prostate Carcinoma	>250	>250
HT-29	Colorectal Adenocarcinoma	>193.2	>200.7
OVCAR-3	Ovarian Cancer	>250	>250
786-0	Renal Cancer	>250	>250
MCF-7	Mammary Adenocarcinoma	>137.1	>153.5
UACC-62	Melanoma	>101.3	>107.8

Different extracts from plants of the genus *Campomanesia* have shown antiproliferative activity.^{41,67-70} Fruit extracts of *C. xanthocarpa*⁶⁷ and *C. adamantium*,⁶⁹ as well as leaf extracts of *C. adamantium*,⁷⁰ exhibited such activity. The dichloromethane extract of the peel and fruits of *C. adamantium* inhibited the proliferation of the MCF-7 and 786-0 cell lines,³⁰ while the hexane extract of the fruits of *C. pubescens* affected the AUCC-62, MCF-7, NCI-ADR/RES, and HT-29 cell lines.⁶⁸ Collectively, these findings reinforce the potential of CSWW and CAWW as promising candidates for cancer treatment.

The differences observed in the antiproliferative responses between CAWW and CSWW may be associated with variations in their phytochemical composition. The biological activity of phenolic compounds is strongly influenced not only by their total concentration but also by their chemical structure, particularly the number and position of hydroxyl groups, degree of polymerization, and conjugation patterns. According to the structure–activity relationship discussed by Rice-Evans *et al.*,⁷¹ flavonoids with specific hydroxylation patterns and conjugated systems display enhanced capacity to modulate oxidative stress and interfere with cellular signaling pathways involved in proliferation and apoptosis. Therefore, the higher flavonoid content observed in CAWW than CSWW may contribute to its comparatively stronger inhibitory effects on certain tumor cell lines.

On the other hand, CSWW showed higher tannin levels and detectable anthocyanins, both recognized for their bioactivity. Tannins may exert antiproliferative effects through protein binding and modulation of enzyme activity,⁷³ while anthocyanins have been reported to interfere with cell cycle progression and induce apoptosis in cancer cells.⁷³

Nevertheless, the biological effects of these compounds depend on their molecular structure and bioavailability. Further chromatographic analyses are necessary to identify the individual compounds responsible for the observed effects. Thus, although the content of phenolic compounds in CAWW and CSWW is comparable, differences in flavonoids, tannins, and anthocyanins likely contribute to the variations in antiproliferative activity. These results emphasize the importance of compositional factors in determining the biological potential of plant-derived extracts and by-products. Overall, the data suggest the samples warrant further fractionation and a more robust biological evaluation to better assess their antiproliferative properties.

4. Conclusion

The CAWW and CSWW demonstrated a relevant chemical composition, retaining expressive levels of secondary metabolites even after essential oil extraction. Both samples presented considerable amounts of total phenolic compounds, flavonoids, and tannins, with significant quantitative differences between species. CAWW showed higher flavonoid and total phenolic contents, whereas CSWW exhibited higher tannin levels and detectable anthocyanins, highlighting species-dependent phytochemical variability.

Photosynthetic pigments were detected in distinct profiles: carotenoids were present in both samples, and chlorophyll α was detected only in CSWW, indicating that hydrodistillation does not completely remove lipophilic plant constituents from the aqueous phase.

In the antiproliferative screening, both wastewaters exhibited inhibitory effects on selected tumor cell lines without affecting the viability of Vero cells, suggesting selective cytotoxicity under the experimental conditions. However, the observed effects should be interpreted as preliminary screening results. The results suggest that fractionation of the extracts monitored by evaluating antiproliferative activity may be promising.

Overall, the findings demonstrate that hydrodistillation wastewater from *Campomanesia* species constitutes a chemically rich by-product containing bioactive compounds. These results contribute to the chemical characterization of the genus and provide a scientific basis for future studies focused on compound identification and detailed biological investigation. In addition, the results contribute to future studies focused on compound identification and detailed biological investigations.

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