Journal of Biotechnology 356 (2022) 8–18

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Low-cost alternative for the bioproduction of bioactive phenolic compounds of callus cultures from *Cereus hildmannianus* (K.) Schum

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ARTICLE INFO

Keywords:
Alternative culture - Cactus - Mass spectrometry - Medicinal herbs - Phytochemicals

ABSTRACT

The aim of this study was to establish a sustainable alternative callus culture of *Cereus hildmannianus* for the production and bioactive determination of phenolic compounds from this species. The conventional callus was cultivated using agar and Murashige and Skoog (MS) medium, while for the alternative culture the agar was replaced with a cotton support covered with filter paper and MS medium (incubated at 32 °C with photoperiod of 16 h), and the morphological characteristics and growth index were assessed (8 weeks). Extracts were obtained by maceration followed by partition, characterized by nuclear magnetic resonance - NMR and ultra-high performance liquid chromatography - UHPLC, quantified (phenolic compounds) by UV-Vis methods, and their antioxidant, antitumor activities, as well as cytotoxicity, were evaluated. The establishment of an alternative callus culture was carried out successfully. Characteristic signals of phenolic compounds were determined by NMR, and 46 compounds with fragment ions were identified using UHPLC analysis. The highest concentrations of phenolic compounds, and greatest antioxidant and antitumor activities, were obtained with the dichloromethane fractions of both callus tissue cultures, which were not cytotoxic. The callus culture from C. hildmannianus has shown promise as a source for the sustainable production of phenolic compounds with antioxidant and antiproliferative activities and thus, has potential use as a natural antitumor product.

1. Introduction

An industrial, economic, and medicinal importance has already been attributed to *Cereus hildmannianus* [syn. *C. peruvianus*] (Cactaceae), which naturally occurs in the south of Brazil where it is often cultivated in gardens (Barros and Nozaki, 2002; Nozaki et al., 1993; Tanaka et al., 2010). The callus culture of this species has been used as a source of important primary (Machado et al., 2004) and secondary metabolites (Oliveira and Machado, 2003; Rocha et al., 2005), which are commonly identified *in natura* plants. Antitumor (Jacomini et al., 2015) and anti-ulcerogenic activities (Jayme et al., 2015) have been reported for the extract obtained from callus tissues of *C. peruvianus*. To our knowledge, however, evaluation of the antioxidant and antitumor activities of extracts enriched with phenolic compounds from the callus culture of *C. hildmannianus* have not yet been reported in the literature.

Currently, studies that emphasize the importance of endemic plants in Brazil and South America are highly necessary given the scarcity of information on the chemical composition of these species, as well as on their nutritional and therapeutic potential (Mota et al., 2019). Some cactus species have been highlighted as sources of phenolic acids (García-Cayuela et al., 2019) and flavonoids (Garcia et al., 2019). These metabolites are of great interest for human health because of their

Abbreviations: DW, dry weight; EC_{50}, effective concentration which scavenges 50% of radical; GI_{50}, cytotoxic activity for 50% of the cells; MS, Murashige and Skoog; NMR, nuclear magnetic resonance; TFC, total flavonoid content; TPC, total phenolic content; UHPLC-ESI-Q-TOF-MS/MS, ultra-high performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry.

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https://doi.org/10.1016/j.jbiotec.2022.07.001
Received 13 May 2022; Received in revised form 1 July 2022; Accepted 11 July 2022
Available online 14 July 2022

antioxidant activity and properties concerning cancer prevention (Milutinović et al., 2019).

Secondary metabolites of plant origin are complex and unique molecules, and due to their extensive biological activities they have been used for centuries for medicinal purposes (Cetin, 2014; Johari and Khong, 2019). However, the production of secondary metabolites *in natura* is generally low since it is dependent on the physiological stage, development of the plant, defense responses, environmental factors, and seasonal variations (Hasanuzzaman et al., 2013; Moses et al., 2013).

Plant biotechnology has emerged as a tool for bioproduction and the study of plant metabolites of interest (Niazian, 2019). This technology largely encompasses gene recombination, which makes it possible to produce new biologically active products (Conner et al., 2014), virus and insect resistant plants (Miao et al., 2019; Zhao et al., 2020), base substances for anti-aging cosmetics (Korkina et al., 2017), flavors and fragrances (Ochoa-Villarreal et al., 2016), natural fungicides (Martínez, 2012), and chemical drugs and recombinant proteins (Efferth, 2019). The *in vitro* culture of plant tissues also represents a renewable, economically viable source for the bioproduction of metabolites of interest (Süntar et al., 2021). The advantageous features of *in vitro* cultures are that they are *i)* maintained under controlled environmental conditions; *ii)* independent of climate or soil change; *iii)* free from microorganisms, insect contamination, and heavy metals from human pollution (Pérez-Molphe-Balch et al., 2015; Vulcano et al., 2008); *iv)* not dependent on seasonal production periods (Kirakosyan et al., 2009); and *v)* able to assure the preservation of plant species. The callus culture is particularly relevant since it can be used as a starter for cell culture establishment to an industrial scale (Lystvan et al., 2018).

The properties of agar that make it the gelling agent of choice for plant tissue culture media are its stability, high clarity, and resistance to metabolism during culture (Norhayati et al., 2011). However, the search for an alternative more economical medium support for callus culture is ongoing. Sustainable alternatives to reduce steps and costs in biotechnological processes (such as the replacement, reuse, or use of fewer reagents) are desirable and of great importance for the planet. Cotton fiber, perlite, and bagasse have been suggested as a replacement for agar, especially in commercial tissue culture laboratories (Moraes-Cerdeira et al., 1995; Saadawy et al., 2008), and currently there is a growing demand for the implementation of sustainable technologies to achieve economic and environmental objectives simultaneously (Lancaster, 2020; Tundo et al., 2000).

Thus, this study aimed to establish an alternative callus culture of *C. hildmannianus* using cotton and filter paper as support, aiming for a more economical bioproduction of bioactive compounds. The chemical characterization of extracts from *C. hildmannianus* was performed by nuclear magnetic resonance (NMR), ultra-high performance liquid chromatography-coupled with electrospray ionization/quadrupole-time-of-flight/mass spectrometry/mass spectrometry (UHPLC-ESI-Q-TOF/MS/MS), the phenolic compounds were quantified by UV-Vis methods, and the antioxidant, antitumor activities, and cytotoxicity were evaluated.

2. Materials and methods

2.1. Plant material

The aerial plant parts (cladodes) of *C. hildmannianus* [syn. *C. peruvianus*] were collected at the State University of Maringá (UEM), Paraná, Brazil, S 23°40′52″, W 51°94′19″, in August of 2018. The species was identified by Dr. Daniela Cristina Zappi, a voucher (no. HUEM 36127) was deposited at the Herbarium of UEM and registered (no. A05B398) in the *Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado* (SisGen).

2.2. Callus cultures: conventional and an alternative culture

Callus tissue culture was induced from hypocotyls of *C. hildmannianus* (Oliveira et al., 1995). The callus tissues of the conventional culture were maintained in Murashige and Skoog (1962) (MS; 20 mL) medium, supplemented with B5 vitamins (Gamborg et al., 1968), 0.8% agar, 3% sucrose, 2,4-dichlorophenoxyacetic acid (2,4-D; 4 mg/L), and *N*-(2 furanylmethyl)− 1*H*-purine-6-amine (kinetin; 4 mg/L) [Patent number BR 102012007410–9].

The alternative culture was established with callus sub-cultured in Petri dishes containing a thin layer of cotton (1 g) covered with qualitative filter paper (0.2 mm; pore 26 μm) and soaked with 3 mL MS medium (supplementary material, Table S1). Conventional and alternative callus tissues were incubated at 32 ± 1 °C, under the photoperiod of 16 h light and 8 h darkness (15 μmol/m^2 s^1 photosynthetic photon flux density, PPFD).

Fresh callus tissues (2 g; corresponding to 100 mg of dry callus tissues) were weighed and transferred to Petri dishes. The growth curves of callus tissues were determined over an 8-week period. The growth index (G*i*) was calculated using Eq. (1) (Krengel et al., 2016).

$$Gi = \frac{DWf - DWi}{DWi} \quad (1)$$

Where, DW_f is the dry weight of final callus tissues, and DW_i is the dry weight of inoculated callus tissues.

2.3. Extraction

Dry cladodes and callus tissues (5 g) were exhaustively extracted with methanol at 25 °C, from which the crude extracts from the plant/cladodes (*E*P), the conventional callus tissues (*E*CC), and the alternative callus tissues (*E*AC) were obtained. The crude extracts were suspended in MeOH:H_2O (1:1, v/v) and successively partitioned with *n*-hexane and dichloromethane (*DCM*), and the remainder was the hydro methanolic (*HM*) fraction. The solvents were removed under reduced pressure at 35 °C to give fractions from the plant (*DCM*P and *HM*P), conventional callus tissues (*DCM*CC and *HM*CC), and alternative callus tissues (*DCM*AC and *HM*AC). Then the crude extracts and fractions were lyophilized and stored, protected from light, at − 20 °C.

2.4. Chemical characterization

2.4.1. ^1H and ^{13}C nuclear magnetic resonance spectroscopy (^1H and ^{13}C NMR)

The NMR spectra were recorded at 298 K using a Bruker Avance III HD spectrometer (Bruker Corporation, USA) operating at 500.0 MHz for the ^1H nucleus and 125.0 MHz for the ^{13}C nucleus, and the samples (10 mg) were dissolved in deuterated methanol (CD_3OD; 0.6 mL). The chemical shifts (δ) were expressed in parts per million (ppm).

2.4.2. Ultra-high performance liquid chromatography (UHPLC-ESI-Q-TOF-MS/MS)

The samples (5 mg) were resuspended in 1 mL methanol (99.9%) and 3 μL of each extract were injected and analyzed using an UHPLC (Shimadzu Nexera X2, Japan) coupled with high-resolution mass spectrometry (HRMS-QTOF; Impact II, Bruker Daltonics Corporation, USA) equipped with an electrospray ionization source (ESI). The capillary voltage was operated in negative and positive ionization mode, set at 4500 V, and with an endplate offset potential of − 500 V. The dry gas parameters were set to 8 L min^{-1} at 200 °C with a nebulization gas pressure of 4 bar. Data were collected from *m/z* 50–1300 with an acquisition rate of 5 spectra per second, and the ions of interest were selected by auto MS/MS scan fragmentation. Chromatographic separation was performed using a C18 column (75 ×2.0 mm i.d.; 1.6 μm; Shimadzu Shim-pack XR-ODS III, Japan). The gradient mixture of

solvents **A** (H_2O) and **B** (acetonitrile with 0.1% of formic acid; v/v) was as follows: 5% **B** 0–1 min, 30% **B** 1–3 min, 95% **B** 3–12 min, maintained at 95% **B** 12–15 min, and 5% **B** 15–17 min, at 40 °C. Ion chromatograms and MS and MS/MS spectra were compared with the literature, identified using a mass spectrometry database and from the error (ppm) calculation using Eq. 2 (Brenton and Godfrey, 2010).

$$\text{Error} = \frac{\text{TM} - \text{EM}}{\text{TM}} x1.000.000 \quad (2)$$

Where, $_{T}M$ is the theoretical exact mass, and $_{E}M$ is the experimental mass.

2.5. Determination of phenolic compounds

2.5.1. Total phenolic content

The total phenolic content (TPC) was determined by the method described by Sousa et al. (2007) with modifications. The samples (40 µL) were mixed with 3.2 mL deionized H_2O, 600 µL Na_2CO_3, and 200 µL of Folin-Ciocalteu reagent. This reaction was incubated for 30 min, and the absorbance was measured in a spectrophotometer (Varian Cary-1E, USA) at 765 nm. A curve with gallic acid was performed (50–250 µg/mL, $r2 = 0.9992$) and the results were expressed as gallic acid equivalents (µg GAE/mg DW).

2.5.2. Total flavonoid content

The total flavonoid content (TFC) was determined by an adapted method described by Pothitirat et al. (2009). The samples (1.5 mL) were mixed with 3.4 mL of an acetic acid methanolic solution (5%, v/v) and 100 µL of $AlCl_3$ methanolic solution (5%, v/v). This reaction was incubated for 30 min and the absorbance was measured at 425 nm. A curve was performed with quercetin (5–100 µg/mL, $r2 = 0.9988$) and the results were expressed as quercetin equivalents (µg QE/mg DW).

2.6. Antioxidant activity assays

2.6.1. Assay for the DPPH radical-scavenging activity

Experimental data on the antioxidant activity was obtained by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Ma et al. (2011). The samples (25 µL) were mixed with 2 mL of the 6.25×10^{-5} mol L^{-1} $DPPH^{\bullet}$ solution for 30 min. The absorbance was measured at 517 nm, and a standard curve with Trolox solution ((±)− 6-hydroxy-2, 5,7,8-tetramethylchroman-2-carboxylic acid) was constructed (200–2000 µmol/L; $r2 = 0.9981$). The results were expressed as µmol Trolox/mg DW (Rufino et al., 2007b) and the effective concentration which scavenges 50% (EC_{50}) of radical DPPH was calculated using Eq. 3 (Lugato et al., 2014). The ascorbic acid was used as a positive control.

$$\text{EC50DPPH} = \frac{\text{A0} - \text{A1}}{\text{A0}} x100 \quad (3)$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the samples.

2.6.2. Assay for the ABTS radical-scavenging activity

This assay was carried out by the 2,2′-azino-*bis*-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method according to Rufino et al. (2007a). The samples (30 µL) were mixed with 3 mL of the $ABTS^{\bullet+}$ solution (5 mL of a 7 mmol/L ABTS solution and 88 µL of a 140 mmol/L potassium persulfate; reaction for 16 h) for 6 min. The absorbance was measured at 734 nm, and a standard curve with Trolox solution was constructed (50–1200 µmol/L; $r2 = 0.9915$). The results were expressed as µmol Trolox/mg DW (Dutra et al., 2019b), and the EC_{50} of radical ABTS was calculated using Eq. 4 (Sridhar and Charles, 2019). The ascorbic acid was used as a positive control.

$$\text{EC50ABTS} = \frac{\text{A0} - \text{A1}}{\text{A0}} x100 \quad (4)$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the samples.

2.6.3. Assay for the ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) method was performed according to Rufino et al. (2006). The samples (100 µL) were mixed with 300 µL deionized H_2O and 3 mL of the FRAP reagent (0.3 M acetate buffer, 10 mM TPTZ (2,4,6-*Tris* (2-pyridyl)-s-triazine) solution, 20 mM ferric chloride solution; 10:1:1, v/v/v) for 30 min at 37 °C. The absorbance was measured at 593 nm, a standard curve with Trolox solution was constructed (100–700 µmol/L; $r2 = 0.9995$), and the results were expressed as µmol Trolox/mg DW (Shi et al., 2019).

2.7. Cytotoxic activity

The human tumor cell lines used were: gastric adenocarcinoma (AGS), colorectal adenocarcinoma (CaCo2), breast adenocarcinoma (MCF-7), and lung carcinoma (NCI-H460); maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 µg/mL). A non-tumor cell line was also tested, green monkey kidney (Vero), which was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, glutamine, and antibiotics. The cells were used only when they had 70–80% confluence.

The samples were dissolved in H_2O to give different concentrations (125–8000 µg/mL), and 10 µL of each sample concentration was incubated with 190 µL of the cell suspension in the wells of a 96-well microplate for 72 h. The microplates were incubated at 37 °C and 5% CO_2, in a humid atmosphere, after checking the adherence of the cells. All cell lines were tested using a concentration of 10,000 cells/well except for the Vero cells that were tested at 19,000 cells/well. After the incubation period, the cells were treated with trichloroacetic acid (TCA 10%, w/v; 100 µL) and incubated for 1 h at 4 °C, washed with water and, after drying, a sulforhodamine B (SRB) solution (0.057%, w/v; 100 µL) was added and the microplates were left to stand at room temperature for 30 min. The plates were washed with acetic acid (1%, v/v) and, after drying, were dissolved with Tris (10 mM, w/v; 200 µL). The absorbance was read at 540 nm (Biotek ELX800, USA).

The results were expressed as a concentration responsible for 50% of cell proliferation inhibition (GI_{50}), and as a positive control, ellipticine was used. The relationship between cytotoxicity in non-tumor cells and tumor cells was determined using the selectivity index (SI).

2.8. Statistical analysis

The assays were performed in triplicate (independent experiments), and the results were presented as mean ± standard deviation (SD). The data were analyzed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA) software. ANOVA and *post-hoc* Tukey's test were used to evaluate the *in vitro* experiments. The results were considered statistically significant for values of $p < 0.05$.

3. Results and discussion

3.1. Establishment of alternative callus tissues

The establishment of an alternative callus tissue culture of *C. hildmannianus* was carried out successfully. The callus tissues adapted well to the new culture method with the absence of agar (Fig. 1**B**). Slower development was observed when compared with the callus tissues cultivated by the conventional method. Regarding color, the cultures remained green like the conventional culture of *C. hildmannianus* callus tissues, however, some callus tissues presented a darker green color. In relation to morphological characteristics, after 8 weeks of culture the alternative callus tissues showed only development in the

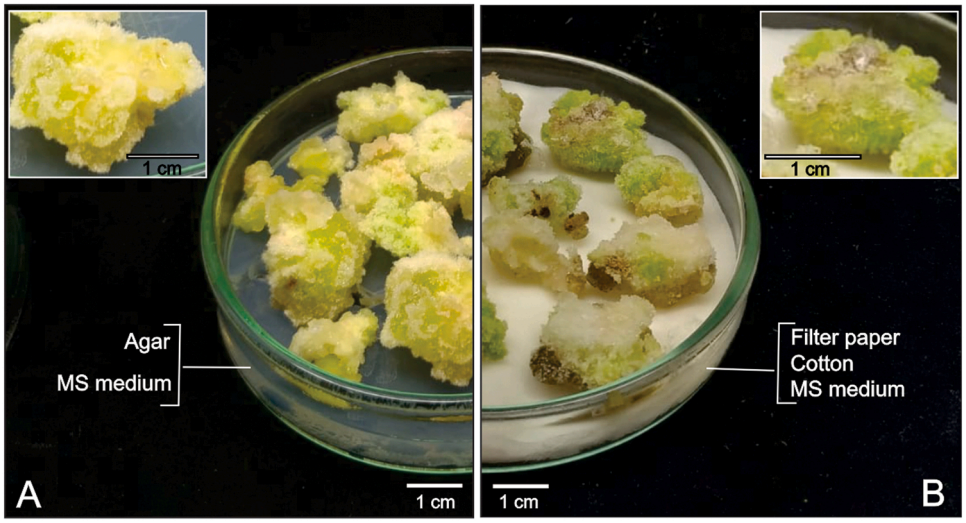

Fig. 1. Photographs of callus tissues of C. hildmannianus after 8 weeks of culture. (**A**) Conventional culture; (**B**) Alternative culture. Bar = 1 cm.

compact texture form, no friable texture callus tissues were observed. The conventional callus tissues meanwhile showed both friable and compact textures, and with greater growth over the Petri dishes (Fig. 1**A**). The friable texture of callus culture is considered embryogenic, which can form organ and shoot tissues (Sharmin et al., 2014).

The culture of callus tissues on cotton requires low-cost support agents, in addition it can be considered sustainable as it saves materials (agar) and its constituents (cotton and filter paper) can be reused in new cycles after drying and sterilizing. Currently, the price of agar for *in vitro* cell culture is about 30 times higher than that of cotton and filter paper; and there is also a large energy requirement for the diffusion of the agar in the culture medium, which is a significant expense in a plant biotechnology laboratory that performs these cultures year-round.

In both the alternative and conventional callus cultures of *C. hildmannianus*, the cell growth curve was characterized by an initial slow stage which was progressive up to 1 week, followed by log phase growth from the 7th to the 52nd day (2st to 6th week), and then exponential growth from the 7th week (52nd to the 60th day). The conventional callus tissues had about a 3.5-fold increase in dry weight (g) and 6.8-fold increase in the growth index (G*i*), while the alternative callus tissues showed about a 1.8-fold increase in dry weight (g) and 5.5-fold increase for the G*i* (Fig. 2**A**, and Fig. 2**B**).

The maximum growth of dry weight, 0.57 ± 0.02 g and 0.22 ± 0.01 g, was achieved after 60 days for the conventional and alternative callus tissues, respectively.

The results demonstrated in the present study, together with those of other studies that used alternatives methods to culture, such as mucilaginous husk from *Plantago ovata* (90.0% of callus generation of *Saccharum* spp.), Psyllium husk (95.8% of root induction from *Ocimum citriodorum*, and 92.7% of reduction of cost), coir fibers (90.0% survival of the seedlings from *Cymbidium pendulum*), corn starch (dry weight of callus from *Nicotiana tabacum* was more than 3 times greater than agar culture, and 47.5% of reduction of cost), and xanthan gum (52.0% of germination, 73.6% of caulogenesis, 56.9% of rhizogenesis from *Albizzia lebbeck*, and 3–13.5 times cheaper than agar culture) (Aggarwal and Nirmala, 2012; Dhawale et al., 2021; Henderson and Kinnersley, 1988; Jain and Babbar, 2006; Saadawy et al., 2008; Tripathi et al., 2021), indicate the potential of this substitution for commercial application.

3.2. NMR characterization

The NMR analyses were performed to characterize the chemical profile of the majority compounds present in the crude extracts (*E*P, *E*CC, and *E*AC) from *C. hildmannianus*. The signals assigned in the ^{1}H NMR spectra (supplementary material Fig. S1) indicated the presence of phenolic acids and flavonoids. This was due to the abundance of phenolic hydroxyl hydrogen (OH) signals (δ 4.0 and 9.0) and the aromatic regions (δ 6.0 and 8.5), in addition to signs of singlets (OCH_3) in the characteristic region of methoxyl of flavonoids (δ 3.5 and 4.0), and the presence of the sugar anomeric proton region, signals close to δ 3.0

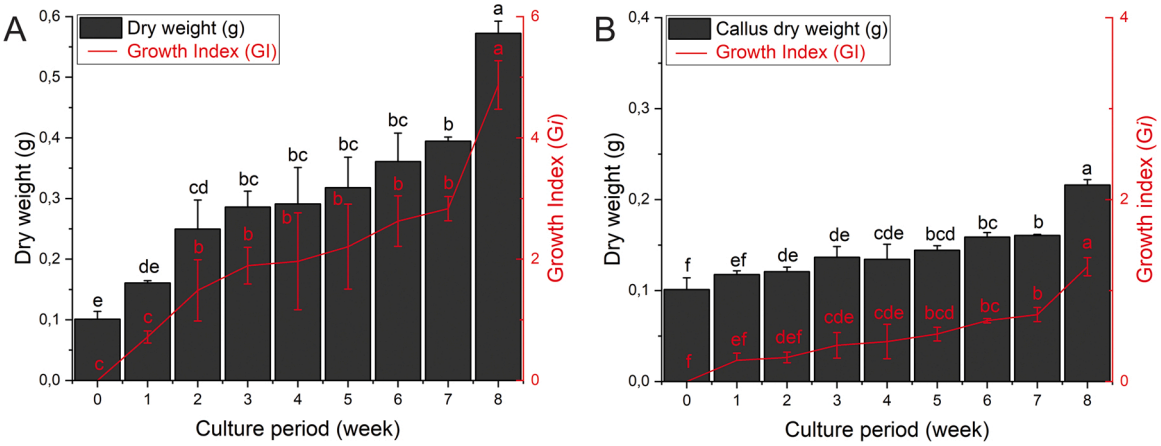

Fig. 2. Growth curve of callus culture of *C. hildmannianus* over 8 weeks of culture. (**A**) Conventional callus culture; (**B**) Alternative callus culture.

and 5.0 (Nerantzaki et al., 2011; Prestes et al., 2012).

The phenolic compounds represent a broad class, commonly constituting substances that have an aromatic ring linked to hydroxyl groups in their structures. According to Pavia et al. (2014), hydrogens bound to aromatic rings are found in a specific region, δ 6.5–8.0, in which few hydrogens are absorbed.

In the ^{13}C NMR spectra the characteristic signals of sugars in the region of δ 70–105 were identified (δ 90.0–105.0 anomeric carbon region), as well as regions of aromatics from δ 110.0–150.0, phenolics above this region of δ 100.0–180.0 (supplementary material Fig. S2), characteristic signals of flavonoid carbonyls at δ 170.0 (Albuquerque et al., 2014), and the region of δ 60.0, which is characteristic of the methoxyl of flavonoids (Dymarska et al., 2018).

Belonging to the group of phenolic compounds, flavonoids are characterized by the presence of two phenolic units linked by 3 carbon atoms, which may contain different patterns of substitution, methylation, hydroxylation, glycosylation, and others (Wan et al., 2011; Zhu et al., 2005). In plants, these compounds can be free, but in general they are in the form of glycosides linked to sugar units (Ayres et al., 2008). As raw extracts were analyzed in this study it is difficult to assign the signals since they constitute a complex mixture of substances. The chemical profile of the extracts from *C. hildmannianus* determined by ^{1}H and ^{13}C NMR showed major and characteristic signs of glycosylated compounds, fatty acids (cuticular compounds), and phenolic compounds.

A greater production of cuticular compounds (hydrocarbons) present in the plant extract was notable, such as wax present in the cladodes from Cactaceae, which has functions in limiting plant water loss through the cuticle (transpiration), UV protection, and defense against pathogens (Sharma et al., 2018; Zeisler-Diehl et al., 2018).

3.3. Total phenolic and total flavonoid contents

Dry cladodes and callus tissues were exhaustively extracted with methanol and the highest crude extract yields were obtained from the alternative callus tissues, followed by conventional callus tissues, both of which showed a higher mass yield than from the cladodes (Table 1). This is possibly due to increased production of wax compounds and fatty acids (cuticular compounds) in the cladodes. The crude extracts were partitioned with *n*-hexane for depigmentation and dichloromethane (*DCM*), and the remainder from this process was the hydromethanolic (*HM*) fraction. With regards to the fractions, the highest yields were obtained with the *HM*, followed by the *DCM*, and the yield of the fractions obtained from both callus tissues were higher than the fractions from the cladodes.

Table 1
Extraction yield, total phenolic content (TPC), and total flavonoid content (TFC) of extracts and fractions of conventional and alternative callus tissues (CC and AC) and plant/cladodes (P) from C. hildmannianus.

Sample	Yield (%)	TPC µg GAE/mg DW	TFC µg QE/mg DW
*E*P	9 ± 2^{c}	756 ± 5^{aA}	90 ± 4^{aB}
*E*CC	26 ± 3^{b}	108 ± 1^{cG}	12 ± 1^{cF}
*E*AC	38 ± 4^{a}	140 ± 1^{bF}	27 ± 3^{bE}
*DCM*P	2 ± 1^{cD}	353 ± 2^{bD}	444 ± 2^{aA}
*DCM*CC	6 ± 1^{bC}	278 ± 6^{cE}	72 ± 2^{bC}
*DCM*AC	9 ± 1^{aC}	384 ± 3^{aC}	39 ± 2^{cD}
*HM*P	73 ± 2^{bB}	543 ± 1^{aB}	6 ± 1^{aF}
*HM*CC	85 ± 2^{aA}	92 ± 6^{cH}	nd
*HM*AC	84 ± 1^{aA}	140 ± 1^{bF}	nd

E, crude extract.
DCM, dichloromethane fraction.
HM, hydromethanolic fraction.
Lowercase letters, statistical difference between extract, and between fraction by one-way ANOVA *post hoc* Tukey's test $p < 0.05$.
Capital letters, statistical difference comparing extracts and fractions by one-way ANOVA *post hoc* Tukey's test $p < 0.05$.
Mean ± standard deviation (sd).
nd, not detected.

The highest total phenolic (TPC) and flavonoid contents (TFC) in the crude extracts were found in the extract from cladodes (*E*P), followed by extracts from alternative callus tissues (*E*AC), and conventional callus tissues (*E*CC). In the fractions the highest TPC was found from the dichloromethane fraction of the alternative callus (*DCM*AC) and the hydromethanolic fraction of the cladodes (*HM*P); and for the TFC, the highest content was found in the dichloromethane (*DCM*P) and hydromethanolic fractions (*HM*P) of the cladodes (Table 1).

In addition to being economical, the alternative callus culture improved the production of bioactive compounds.

Phenolic compounds are the most widely occurring groups (Asadi-Samani et al., 2019), and are ubiquitously distributed phytochemicals found in most plant tissues, including fruits and vegetables (Laura et al., 2019). It is estimated that there may be more than 2000 types of phenolic compound classes, making it difficult to develop an ideal extraction method and so several solvents often need to be used for extraction (Osmić et al., 2019).

The stress *in natura* plants may be responsible for enhancing the bioproduction of phenolic compounds (Ksouri et al., 2008) through the activation of chalcone synthase, a key enzyme of the flavonoid biosynthesis pathway (Dao et al., 2011). The flavonoids may occur in plants in the form of glycosides in several glycosidic combinations (Sati et al., 2019).

The detection of these important secondary metabolites (phenolic compounds) from *C. hildmannianus* cladodes and *in vitro* callus culture are promising for the exploitation of this species by the food and pharmaceutical industries.

3.4. Identification of phenolic compounds by UHPLC-ESI-Q-TOF-MS/MS

Chemical investigation of extracts and fractions of *C. hildmannianus* resulted in the putative identification of forty-six compounds. The samples were analyzed by high-resolution mass spectrometry, and the identification was proposed after a review of the genus *Cereus* and family Cactaceae (Astello-García et al., 2015; Cabañas-García et al., 2019; Hernández-García et al., 2019; Kumar et al., 2018; Slimen et al., 2017), in addition to mass error value (supplementary material, Table S2, and Fig. S3).

The analysis of the extracts and fractions provided the characterization of thirty-four phenolic and organic acids and their derivatives, as well as twelve flavonoids (eight glycosylated flavonoids). The comparative study by UHPLC-ESI-Q-TOF-MS/MS of the samples showed an excellent production of phenolic compounds by the callus culture, compared with the plant (Table 2).

Some compounds were found to be tissue-specific. In the plant/cladode these compounds were: dihydroxy methoxy butanoic acid (**5**), dalbergioidin (**20**), catechin (**21**), kaempferol 3-*O*-rhamnopyranoside (1→2)-glucopyranoside]− 7-*O*-rhamnopyranoside (**44**), and isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (**45**).

The compounds identified only in conventional callus tissues were: vanillic acid (**9**), quinic acid (**14**), ethyl gallate (**16**), and acacetin (**19**); and those only in alternative callus tissues were rhamnetin (**23**) and protocatechuic acid-hexoside (**24**). In both callus tissues, ethyl benzoate (**6**), *p*-coumaric acid (**8**), ferulic acid (**15**), glucoheptonic acid (**17**), hydroxybenzoic acid-hexose (**22**), kaempferol-3,7,4′-trimethyl ether (**25**), 2-isoferulic acid piscidic-1-methyl ester (**35**), D-xylofuranose tetradecyl glycoside (**37**), quercetin 3-*O*-rutinoside (rutin) (**41**), hesperidin (**42**), and isorhamnetin 3-*O*-sophoroside-7-*O*-rhamnoside (**46**) were identified. Callus cultures showed greater bioproduction and diversity of phenolic compounds in relation to the cladodes. This is possibly due to the chemical composition and concentration of the culture medium, such as the carbon source (Paiva Neto and Otoni, 2003), as the callus tissue cultures can easily access these components, while *in natura* they may be more scarce and can be dependent on seasonality and other

Table 2
Putative identification of compounds by UHPLC-ESI-Q-TOF-MS/MS in extracts and fractions of conventional and alternative callus tissues (CC and AC) and cladodes (P) from C. hildmannianus.

Compound	#	Mf	TM (*m/z*)	Plant			Conventional callus tissues			Alternative callus tissues			Class
				EP	*DCMP*	*HMP*	*ECC*	*DCMCC*	*HMCC*	*EAC*	*DCMAC*	*HMAC*	
Succinic acid	**1**	$C_4H_6O_4$	118.0266		✓	✓	✓	✓	✓		✓	✓	Carboxylic acid
Benzoic acid	**2**	$C_7H_6O_2$	122.0368	✓	✓	✓	✓		✓	✓		✓	Carboxylic acid
Malic acid	**3**	$C_4H_6O_5$	134.0215	✓		✓	✓	✓	✓	✓	✓	✓	Carboxylic acid
Salicylic acid	**4**	$C_7H_6O_3$	138.0336				✓	✓	✓	✓		✓	Carboxylic acid
Dihydroxy methoxy butanoic acid	**5**	$C_5H_{10}O_5$	150.0528			✓							Carboxylic acid
Ethyl benzoate	**6**	$C_9H_{10}O_2$	150.0680					✓	✓			✓	Carboxylic acid
Vanillin	**7**	$C_8H_8O_3$	152.0473		✓			✓			✓	✓	Phenolic aldehyde
p-Coumaric acid	**8**	$C_9H_8O_3$	164.0473				✓			✓			Phenolic acid
Vanillic acid	**9**	$C_8H_8O_4$	168.0423					✓					Phenolic acid
Gallic acid	**10**	$C_7H_6O_5$	170.0215			✓			✓			✓	Phenolic acid
2-isopropylmalic acid	**11**	$C_7H_{12}O_5$	176.0684			✓			✓			✓	Carboxylic acid
Propyl paraben	**12**	$C_{10}H_{12}O_3$	180.0786								✓		Phenolic acid
Azelaic acid	**13**	$C_9H_{16}O_4$	188.1049	✓	✓		✓	✓		✓	✓		Phenolic acid
Quinic acid	**14**	$C_7H_{12}O_6$	192.0634					✓					Phenolic acid
Ferulic acid	**15**	$C_{10}H_{10}O_4$	194.0579				✓		✓	✓	✓	✓	Phenolic acid
Ethyl gallate	**16**	$C_9H_{10}O_5$	198.0528					✓					Phenolic acid
Glucoheptonic acid	**17**	$C_7H_{14}O_8$	208.0583				✓	✓	✓	✓	✓	✓	Phenolic acid
Piscidic acid	**18**	$C_{11}H_{12}O_7$	256.0583	✓		✓	✓	✓	✓	✓	✓	✓	Phenolic acid
Acacetin	**19**	$C_{16}H_{12}O_5$	284.0685					✓					Flavone
Dalbergioidin	**20**	$C_{15}H_{12}O_6$	288.0634		✓								Isoflavonoids
Catechin	**21**	$C_{15}H_{14}O_6$	290.0790		✓								Flavan-3-ol
Hydroxybenzoic acid-hexose	**22**	$C_{13}H_{16}O_8$	300.0845						✓	✓	✓	✓	Phenolic acid
Rhamnetin	**23**	$C_{16}H_{12}O_7$	316.0583								✓		Flavonol
Protocatechuic acid-hexoside	**24**	$C_{13}H_{16}O_9$	316.0794									✓	Phenolic acid
kaempferol 3,7,4′-trimethyl ether	**25**	$C_{18}H_{16}O_6$	328.0946					✓			✓		Flavonol
Tianshic acid	**26**	$C_{18}H_{34}O_5$	330.2406	✓	✓		✓	✓		✓	✓		Phenolic acid
Cyclohexanecarboxylic	**27**	$C_{14}H_{24}O_{10}$	352.1369	✓		✓	✓		✓	✓	✓	✓	Carboxylic acid
Compound	#	Mf	TM (*m/z*)	Plant			Conventional callus culture			Alternative callus culture			Class
				EP	*DCMP*	*HMP*	*ECC*	*DCMCC*	*HMCC*	*EAC*	*DCMAC*	*HMAC*	
Chlorogenic acid	**28**	$C_{16}H_{18}O_9$	354.0950	✓									Phenolic acid
Ferulic acid-hexose	**29**	$C_{16}H_{20}O_9$	356.1107	✓		✓	✓	✓	✓	✓	✓	✓	Phenolic acid
Caffeic acid derivative	**30**	$C_{18}H_{18}O_9$	378.0950			✓	✓	✓	✓	✓	✓	✓	Phenolic acid
Sinapic acid-hexoside	**31**	$C_{17}H_{22}O_{10}$	386.1212	✓			✓		✓	✓		✓	Phenolic acid
Dihydroxybenzoic acid-*O*-dipentoside	**32**	$C_{17}H_{22}O_{12}$	418.1111			✓			✓		✓	✓	Phenolic acid
Benzyl alcohol-dihexose	**33**	$C_{19}H_{28}O_{11}$	432.1631	✓		✓	✓		✓			✓	Phenolic acid
Propanedioic acid, 5-[[2-[(6-deoxy-α-L-galactopyranosyl) oxy]-cyclohexyl]-oxy]-pentyl]	**34**	$C_{20}H_{34}O_{10}$	434.2151			✓							Carboxylic acid
2-Isoferulic acid piscidic-1-methyl ester	**35**	$C_{22}H_{22}O_{10}$	446.1212						✓	✓		✓	Phenolic acid
Lucuminic acid	**36**	$C_{19}H_{26}O_{12}$	446.1424	✓					✓	✓		✓	Phenolic acid
D-xylofuranose tetradecyl glycoside	**37**	$C_{22}H_{42}O_9$	450.2828					✓			✓		Alkyl pentoside
Ferulic acid derivative	**38**	$C_{21}H_{32}O_{13}$	492.1842			✓							Phenolic acid
Ferulic acid-dihexose	**39**	$C_{22}H_{30}O_{14}$	518.1635				✓		✓			✓	Phenolic acid
Kaempferol 3-*O*-rutinoside	**40**	$C_{27}H_{30}O_{15}$	594.1585	✓		✓	✓		✓	✓		✓	Flavonol
Quercetin 3-*O*-rutinoside	**41**	$C_{27}H_{30}O_{16}$	610.1533									✓	Flavonol
Hesperidin	**42**	$C_{28}H_{34}O_{15}$	610.1897				✓		✓	✓		✓	Flavanone
Narcissin	**43**	$C_{28}H_{32}O_{16}$	624.1690	✓		✓	✓		✓	✓		✓	Flavonol
Kaempferol 3-*O*-rhamnopyranoside (1→2)-glucopyranoside]− 7-*O*-rhamnopyranoside	**44**	$C_{33}H_{40}O_{19}$	740.2164	✓		✓							Flavonol
Isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside)	**45**	$C_{34}H_{42}O_{20}$	770.2269			✓							Flavonol
Isorhamnetin 3-*O*-sophoroside-7-*O*-rhamnoside	**46**	$C_{34}H_{42}O_{21}$	786.2218				✓		✓	✓		✓	Flavonol
Number of phenolic, organic acids and derivatives				11	5	14	16	15	20	16	15	22	
Number of flavonoids				3	2	4	4	2	4	4	2	5	
Total number of phenolic compounds				14	7	18	20	17	24	20	17	27	

Mf, molecular formula.
TM, theoretical exact mass.
m/z, mass-to-charge ratio.
✓, identification in sample.

environmental factors.

The phenolic compounds, ferulic (**15**), *p*-coumaric (**8**), caffeic and sinapic acids, are molecular precursors of lignin biosynthesis (Dixon and Barros, 2019; Sakamoto et al., 2020), and lignin is essential for the growth of *C. hildmanniuanus* cladodes *in natura* and callus tissues *in vitro*. In addition, these are important molecules in the formation of flavonoids, responsible for the chemical defense of plants.

The chemical characterization of these molecules was also done by the fragmentation profile compared with the literature to confirm the compounds; the fragmentation pathway of some compounds that were not found in the literature was also proposed and these are illustrated in Fig. 3. The compounds identified are: **A**) Rhamnetin (**23**), precursor ion identified at *m/z* 297.0390 [M-H_2O-H]$^-$ and its fragment ions at *m/z* 284.0293 produced by the loss of a methoxyl and at *m/z* 269.0453 produced by the loss of a hydroxyl. **B**) Sinapic acid-hexose (**31**), precursor ion identified at *m/z* 385.1116 [M-H]$^-$ and its fragment ions at *m/z* 223.0594 with a loss of *C*-glycoside (-162.0522), in addition, an ion fragment was observed at *m/z* 179.0559 referring to the deprotonated hexose (-206.0557), which was attributed to the loss of a sinapic acid. **C**) Narcissin (**43**), is being reported for the first time in the genus *Cereus*, with a precursor ion identified at *m/z* 623.1603 [M-H]$^-$, originating the aglycone, corresponding to the flavonoid isorhamnetin; the heterolytic cleavage produced an intense fragment ion at *m/z* 315.0482 (−326.1213). The mechanism for generating this ion involves the transfer of a proton from the sugar to the glycosidic oxygen atom and the heterolytic cleavage of the hemiacetal glycosidic bond. Furthermore, the ion fragment at *m/z* 299.0176 was produced by the loss of a di-*O*-glycoside group, and the cryoseriol structure can be confirmed as the resulting fragment. **D**) Kaempferol 3-*O*-rhamnopyranoside (1→2)-glucopyranoside]− 7-*O*-rhamnopyranoside (**44**), precursor ion identified at *m/z* 739.2044 [M-H]$^-$, and its fragment ions at *m/z* 447.2907 produced by successive losses of 146 u, di-*O*-rhamnoside, and *O*-glycoside residues (-291.9137), then the loss of 162 u as a glucose residue generating a fragment ion at *m/z* 285.0368.

The identification of flavonoids in callus tissues from *C. hildmannianus* corroborate those found in other cacti species, such as kaempferol 3,7,4′-trimethyl ether (**25**) in *Opuntia ficus-indica* (Avila-Nava et al., 2014; Lee et al., 2003); and kaempferol-3-*O*-rutinoside (**40**), quercetin-3-*O*-rutinoside (rutin) (**41**), and isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside) (**45**) in *Opuntia* spp. (Astello-García et al., 2015; Guevara-Figueroa et al., 2010).

3.5. Antioxidant activity

The plant tissue culture was a platform to investigate the antioxidant activity of *C. hildmaniannus* in a controlled environment (Niazian, 2019) since there is a lack of data regarding the antioxidant activity of callus tissues from this species in the literature. This was of particular interest as the phenolic profile of *C. hildmannianus*, determined by NMR and UHPLC-ESI-Q-TOF-MS/MS, already showed the presence of compounds with antioxidant potential, such as the vanillin, acacetin, kaempferol 3-*O*-rutinoside, and narcissin (Hadžifejzović et al., 2013; More et al., 2012; Ortega-Valencia et al., 2019; Rosidah et al., 2008).

The extracts and fractions of cladodes and callus tissues from *C. hildmannianus* presented antioxidant activity by all methodologies evaluated (DPPH, ABTS, and FRAP; Table 3). The greatest antioxidant potential was obtained with the dichloromethane fractions from alternative callus tissues (*DCM*AC) per Trolox equivalent and EC_{50} from the DPPH and ABTS assays, followed by the conventional callus tissues (*DCM*CC). The FRAP assay also indicated better activity with *DCM*AC (Table 3).

This potential antioxidant activity with alternative callus tissues is probably due to the higher production of phenolic acids present in these tissues, and with a higher concentration in their *DCM* fractions (Table 1). These results suggest promising antioxidant activity from extracts of the species *C. hildmannianus*, and that the alternative callus tissues represent a viable and sustainable source for the bioproduction of phenolic acids and flavonoids with possible industrial applications.

These results are in accordance to those described by Dutra et al. (2018), where the hydroalcoholic extract of the *C. jamacaru* plant, which is rich in phenolic compounds, presented activity with an EC_{50} of 0.42 and 0.27 mg/mL (DPPH and ABTS). Dutra et al. (2019a) reported a greater antioxidant activity in the hydroalcoholic extracts of *C. jamacaru* cladodes with fruiting in the DPPH and ABTS assays. The cladode extract cultivated *in vitro* of *Turbinicarpus valdezianus* had potential radical inhibition of ABTS at 91.7%, and of DPPH at 65.0% (Kim et al., 2019).

According to the literature, several studies have already demonstrated antioxidant activity in the callus tissue culture of some species, attributing this activity to phenolic acids and flavonoids (Ali et al., 2018; Yang et al., 2019).

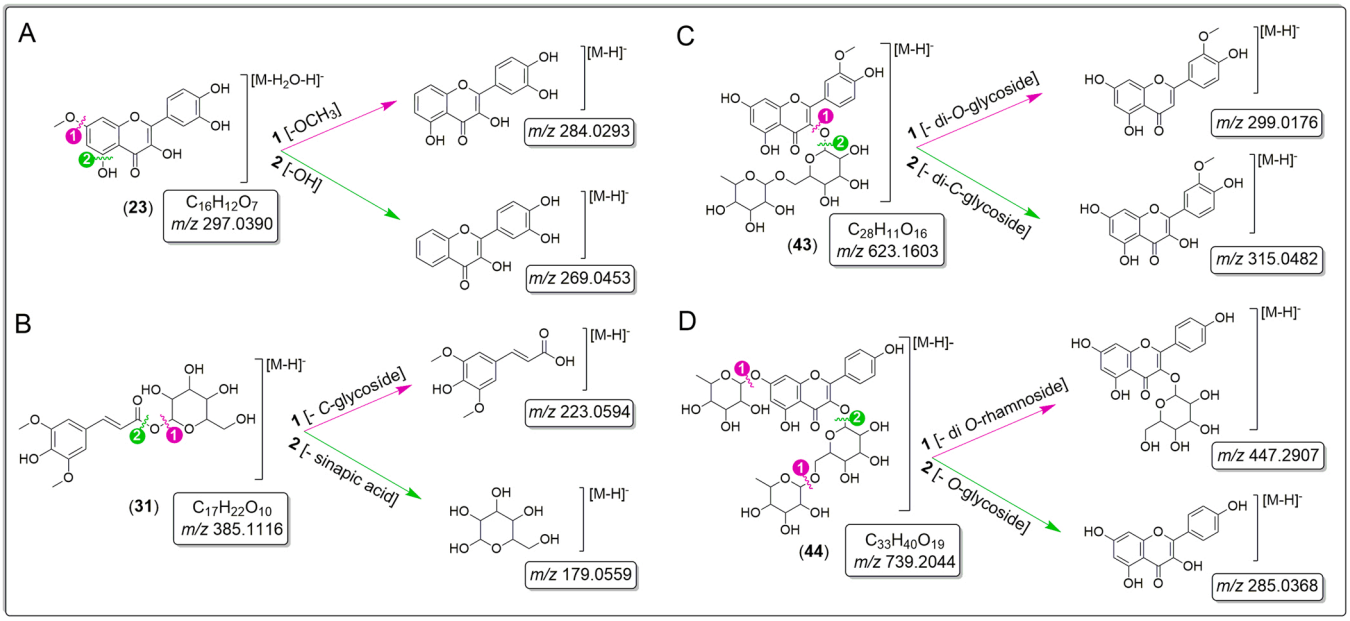

Fig. 3. Fragmentation proposals of compounds identified by UHPLC-ESI-Q-TOF-MS/MS. (**A**) Rhamnetin (**23**); (**B**) Sinapic acid-hexoside (**31**); (**C**) Narcissin (**43**); and (**D**) Kaempferol 3-O-rhamnopyranoside (1→2)-glucopyranoside]− 7-O-rhamnopyranoside (**44**).

Table 3
Antioxidant activity of extracts and fractions of conventional and alternative callus tissues (CC and AC) and cladodes (P) from *C. hildmannianus*.

Sample	DPPH		ABTS		FRAP
	µmol Trolox/mg DW	EC_{50} mg/mL	µmol Trolox/mg DW	EC_{50} mg/mL	µmol Trolox/mg DW
*E*P	223 ± 2^{cE}	nd	162 ± 1^{aCD}	11 ± 0.4^{Ac}	125 ± 2^{aB}
*E*CC	236 ± 9^{abCDE}	nd	146 ± 3^{bE}	nd	65 ± 7^{bCD}
*E*AC	249 ± 5^{aC}	nd	119 ± 1^{cF}	21 ± 1^{bG}	41 ± 1^{cE}
*DCM*P	267 ± 4^{bB}	nd	158 ± 3^{cD}	13 ± 0.1^{cE}	19 ± 3^{cF}
*DCM*CC	311 ± 1^{aA}	15 ± 0.1^{aA}	171 ± 2^{bC}	8 ± 0.1^{bB}	114 ± 8^{bB}
*DCM*AC	328 ± 11^{aA}	15 ± 0.2^{aA}	206 ± 9^{aA}	7 ± 0.1^{aA}	210 ± 4^{aA}
*HM*P	230 ± 5^{bDE}	nd	184 ± 3^{aB}	13 ± 0.1^{bE}	66 ± 1^{abCD}
*HM*CC	188 ± 4^{cF}	nd	116 ± 5^{bF}	14 ± 010^{cF}	61 ± 5^{bD}
*HM*AC	241 ± 3^{aCD}	19 ± 0.1^{aB}	100 ± 2^{cG}	12 ± 0.2^{aD}	74 ± 1^{aC}
Ascorbic acid	2356 ± 1	0.2 ± 0.0	3388 ± 4	0.1 ± 0.0	*

E, crude extract.
DCM, dichloromethane fraction.
HM, hydromethanolic fraction.
Lowercase letters, statistical difference between extract and between fraction by one-way ANOVA *post hoc* Tukey's test $p < 0.05$.
Capital letters, statistical difference comparing extracts and fractions by one-way ANOVA *post hoc* Tukey's test $p < 0.05$.
Mean ± standard deviation (sd).
EC_{50}, effective concentration which scavenges 50% of radical.
* , not tested.
nd, not detected.

The antioxidant potential of plants is important to relieve pain, inflammation (Bursal et al., 2019), cardiovascular disease, and cancer (Adil et al., 2019), as free radicals and reactive oxygen play an important role in carcinogenesis by inducing DNA damage (Lobo et al., 2010). Chain breaking antioxidants prevent this damage by interfering with free radical propagation cascades (Matkowski, 2008).

3.6. Cytotoxic activity

All extracts and fractions of cladodes and conventional and alternative callus tissues from *C. hildmanninaus* showed activity against one or more tumor cell lines. This evidence may be related to the phenolic compounds, thus the antitumoral activity of phenolic-rich extracts is reported for the first time for this species. The most promising GI_{50} results were obtained with the *DCM* fractions; from the cladodes (*DCM*P) against MCF-7 (177 µg/mL) and AGS (199 µg/mL), and alternative callus tissues (*DCM*AC) against AGS (193 µg/mL) and CaCo2 cells (203 µg/mL). The crude extracts (*E*P and *E*AC) showed activities against CaCo2 and NCI-H460, while the *HM* fractions were only against CaCo2 (*HM*P and *HM*CC) (Table 4).

None of the extracts or fractions of *C. hildmannianus* had cytotoxic activity against the non-tumor cells tested (Vero: GI_{50} <400 µg/mL; Table 4). The obtained results are positive, showing a potential application as an antitumor product, since the majority of substances tested with activity against tumor cells usually also show some cytotoxicity against healthy cells (Chari, 2008; Domińska et al., 2022).

According to Jacomini et al. (2015), the lipidic extract of callus culture from *C. peruvianus* (syn. *C. hildmannianus*) is active against CaCo2 cells with a GI_{50} of 250 µg/mL. Furthermore, the hydroalcoholic extract of *C. jamacaru* has been reported to cause significant decrease of sarcoma 180 cells (murine sarcoma) (Dutra et al., 2018), while the pulp extracts of *C. jamacaru* subsp. *jamacaru*, did not show cytotoxic activity against the L929 mouse fibroblast cell line (Santos et al., 2021). Several studies have reported cytotoxic activity of phenolic acids and flavonoids in different cell lines (Radünz et al., 2020; Sobral et al., 2017).

The selectivity index (SI) is an excellent calculation to assess the selective power of samples against tumor over non-tumor cells, and this number is expressive when near or ≥ 2.0 (Suffness and Pezzuto, 1991). Given this aspect, the results obtained in Table 4 revealed that the dichloromethane fractions were the greatest, highlighting the SI of *DCM*PC against AGS (2.0), MCF-7 (2.3), and NCI-H460 (1.8); *DCM*CAC against MCF-7 (1.8) and NCI-H460 (1.6); and *DCM*ACC against AGS (2.1) and CaCo2 (1.9).

Table 4
Cytotoxic activity of extracts and fractions of conventional and alternative callus tissues (CC and AC) and cladodes (P) from *C. hildmannianus*.

Sample	Cytotoxic activity (GI_{50} µg/mL)								
	AGS	SI	CaCo2	SI	MCF-7	SI	NCI-H460	SI	Vero
*E*P	> 400		332 ± 13^{cA}	1.2	> 400		389 ± 6^{dB}	1.0	> 400
*E*CC	> 400		> 400		> 400		> 400		> 400
*E*AC	> 400		310 ± 7^{bcA}	1.3	> 400		> 400		> 400
*DCM*P	198 ± 14^{aAB}	2.0	> 400		177 ± 11^{aA}	2.3	218 ± 2^{aC}	1.8	> 400
*DCM*CC	250 ± 11^{bC}	1.6	304 ± 8^{bcD}	1.3	220 ± 6^{aA}	1.8	248 ± 7^{bB}	1.6	> 400
*DCM*AC	193 ± 8^{aA}	2.1	213 ± 19^{aB}	1.9	> 400		273 ± 4^{cC}	1.5	> 400
*HM*P	> 400		293 ± 18^{bA}	1.4	> 400		> 400		> 400
*HM*CC	> 400		368 ± 7^{dA}	1.1	> 400		> 400		> 400
*HM*AC	> 400		> 400		> 400		> 400		> 400
Ellipticine	1.23 ± 0.03	1.2	1.21 ± 0.02	1.2	1.02 ± 0.02	1.2	1.01 ± 0.01	1.4	1.4 ± 0.1

E, crude extract.
DCM, dichloromethane fraction.
HM, hydromethanolic fraction.
AGS, gastric adenocarcinoma.
CaCo2, colorectal adenocarcinoma.
MCF-7, breast adenocarcinoma.
NCI-H460, lung carcinoma.
Vero, green monkey kidney.
Lowercase letter, statistical difference between extracts and fractions against a single cell line.
Capital letter, statistical difference comparing each extract/fraction in relation to all cell lines by Tukey test $p < 0.05$.
Mean ± standard deviation (sd).
GI_{50}, cytotoxic concentration for 50% of the cells.
SI, selectivity index.

Phenolic compounds are an important group of secondary metabolites that have been shown to be effective against tumor cells (Liu et al., 2020). Among the phenolic compounds, the activity of vanillin (Anand et al., 2019), acacetin (Kim et al., 2014), narcisin (Alhozaimy et al., 2017), rutin (Alonso-Castro et al., 2013), and rhamnetin (Nisari et al., 2020) can be highlighted. The present results align with the literature, suggesting that the antioxidant and antitumor activities are related to the presence of these compounds in fractions dichloromethane (*DCM*) of species *C. hildmannianus*.

These results show that extracts and fractions of *C. hildmannianus* are promising alternatives against several tumor cell lines, especially against CaCo2 and NCI-H460.

4. Conclusion

The alternative callus culture of *C. hildmannianus* using cotton as support was successfully established and offers new possibilities of using a low-cost material as an agar alternative. It was demonstrated that this alternative callus culture produced phenolic compounds, and this study is the first report on the antioxidant activity and the NMR and UHPLC-MS/MS identification of phenolic compounds of the callus tissues of this species. The greatest antiproliferative activity was obtained with dichloromethane fractions from both the alternative and conventional callus tissue cultures of *C. hildmannianus*, in addition to not presenting cytotoxicity against non-tumor cells.

The *in vitro* cultures and cladodes of *C. hildmannianus* represents a sustainable and promising source of phenolic compounds with probable commercial application, such as in natural anti-aging and antitumor products.

CRediT authorship contribution statement

Éverton da Silva Santos: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Aline Savam**: Methodology. **Márcia Regina Pereira Cabral**: Investigation, Methodology. **Juliana Cristina Castro:** Methodology, **Sandra Aparecida de Oliveira Collet**: Conceptualization, Investigation, Methodology. **Maria de Fatima Pires da Silva Machado**: Conceptualization, Investigation, Methodology, Writing – review & editing. **Filipa Mandim**: Methodology. **Ricardo C. Calhelha**: Methodology. **Lillian Barros:** Investigation, Methodology. **Arildo José Braz de Oliveira:** Formal analysis, Investigation, Methodology, Writing – review & editing, Supervision. **Regina Aparecida Correia Gonçalves**: Conceptualization, Writing – review & editing, Supervision.

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.

Acknowledgments

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) - Finance Code 001, Brazilian National Council for Scientific and Technological Development and to Complex of Research Support Centers of the State University of Maringá (COMCAP-UEM) for the facilities, and Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES to the CIMO (UIDB/00690/2020). L. Barros and R. Calhelha thank the national funding by FCT, P.I., through the institutional and individual scientific employment program-contract for their contracts; F. Mandim thanks for their PhD grant.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbiotec.2022.07.001.

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