Journal of CO2 Utilization 49 (2021) 101563

Contents lists available at ScienceDirect

Journal of CO2 Utilization

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

Use of supercritical CO_2 to obtain *Baccharis uncinella* extracts with antioxidant and antitumor activity

Aline Machado Lucas [a,*], Amerícia F.M.L. Bento [a], Rubem Mário Figueiró Vargas [b], Thamiris Becker Scheffel [c], Liliana Rockenbach [c], Fernando Mendonça Diz [b], Angélica Regina Capellari [c], Fernanda B. Morrone [c], Eduardo Cassel [b]

[a] Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Escola Politécnica, Porto Alegre, RS, Brasil
[b] Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Escola Politécnica, Programa de Pós Graduação em Engenharia e Tecnologia de Materiais, Porto Alegre, RS, Brasil
[c] Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Escola de Ciências da Saúde e da Vida, Programa de Pós Graduação em Biologia Celular e Molecular, Porto Alegre, RS, Brasil

ARTICLE INFO

Keywords:
Supercritical fluid extraction
Antioxidant activity
Antitumoral activity

ABSTRACT

The aim of the present study is to evaluate the antioxidant activity (AA) of different fractions of the supercritical extract of *Baccharis uncinella*, in addition to investigating and relating AA to the potential antitumor action in bladder cancer (T24) and glioblastoma cells (U87) and also its toxicity against normal cells (Vero) for the fraction with the highest percentage of free radical scavenging (% FRS). The extracts were obtained sequentially by extraction with supercritical CO_2 (150 and 200 bar and 60 °C) using ethanol as a co-solvent. The fractionation was carried out by column chromatography with hexane, dichloromethane, ethyl acetate and methanol as eluents. AA was tested by the DPPH free radical scavenging method and cell toxicity tests were performed by the MTT assay. The methanolic fraction had the highest AA, with %FRS of 86.7 %, very close to the antioxidant standard used in the test, quercetin, with 87.9 %. The methanolic fraction was able to reduce cell viability to less than 50 % at a concentration of 158.8 μg/mL to T24, 213.9 μg/mL to U87 and 702.5 μg/mL to Vero cells. According to the results, the methanolic fraction of the supercritical extract of *B. uncinella* has an antiproliferative effect for the tumor cells tested, with less effects on the normal cells evaluated.

1. Introduction

The *Baccharis* genus includes more than 500 species distributed from the United States of America to Argentina, 90 % of which occur in South America. In Brazil, there are approximately 120 described species [1]. Its economic importance is mainly from combating soil erosion and in traditional medicine [1,2]. Previous studies have reported the presence of phenolic compounds such as flavonoids and phenolic acids, diterpenes and saponins in their non-volatile extracts [3–5]. Its described biological and pharmacological properties are antiparasitic, anti-inflammatory, analgesic, antioxidant and antibacterial [3,4,6,7].

In folk medicine *Baccharis* are widely used in the form of tea for gastrointestinal problems. Such diseases are often attributed to the oxidative process of stomach tissues and other organs of the digestive system [8,9]. The proof of the antioxidant activity of *Baccharis* extracts [4] can be an empirical justification for its use in gastrointestinal diseases, even without scientific evidence.

Obtainment of plant extracts generally occurs by macerating the plant material with organic solvents [8,10,11]. This extraction method leads to a later step of removing the remaining solvent [12,13,9]. An alternative method for obtainment is through extraction with supercritical fluid, using CO_2 as a solvent. The extract obtained by this technique is free of residual solvent and has been shown to be a selective process for phenolic compounds, according to reports from previous studies [4,14]. The solvent most used as a supercritical fluid is CO_2 (carbon dioxide) because it is non-toxic and has supercritical conditions that are easy to obtain. Supercritical CO_2 has low polarity, thus the increase in pressure, as well as the use of co-solvents, induces an increase in the polarity of the system, helping to extract different compounds from the plant matrix [15].

The techniques of fractionation of plant extracts are usually based on the difference in polarity and solubilization power of the compounds in

* Corresponding author at: Av. Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brasil.
E-mail address: aline.lucas@pucrs.br (A.M. Lucas).

https://doi.org/10.1016/j.jcou.2021.101563
Received 12 February 2021; Received in revised form 9 April 2021; Accepted 2 May 2021
Available online 13 May 2021

organic solvents. Column chromatography separation is one of the most used techniques, being considered a preparative method that aims to isolate major compounds and/or decrease the number of compounds for further analysis, separating the extract into several fractions. Its principle of separation is based on the phenomenon of adsorption, which can be defined as a process in which a substance (gas, liquid or solid) is retained on the surface of a solid [16].

Various substances produced by the secondary metabolism of organisms are being used in the pharmaceutical industry for the production of medicines and cosmetics. Such substances are responsible for species protection and environmental mechanisms [17] and are increasingly being studied in order to find a beneficial and sustainable use for human beings. Native plant species are among the most studied, due to their availability and sustainability, as well as being a way of valuing what each region can offer. One of the most researched properties in recent years in plant extracts is antitumor activity [18–21]. Conventional chemotherapies, in addition to being toxic to tumor cells are also extremely toxic to normal cells, causing a series of side effects [22–26].

Cancer, along with cardiovascular diseases, is one of the main causes of mortality worldwide and, therefore, the subject of several studies aimed at the development of drugs for its treatment [27]. Currently, cancer treatment strategies involve agents that inhibit the initiation of the carcinogenic process or that inhibit the proliferation and progression of the disease [28,29]. Chemotherapy involves the administration of a synthetic compound or one or more naturally occurring chemical agents with potential antineoplastic action for various types of neoplasms, including bladder cancer and glioblastoma [27,29–31].

The main chemotherapy used in tumors treatment is cisplatin. Cisplatin is effective against several types of cancers, including carcinomas, lymphomas and sarcomas. Its action is related to the ability to associate with the DNA, causing damage and leading cancer cells to apoptosis [32–34]. One of the main problems presented by chemotherapy drugs is toxicity in healthy cells. To minimize adverse effects, combinations of drugs and even plant extracts have been tested [23,35].

The aim of the present study is to evaluate the antioxidant potential of fractions of the supercritical extract of *B. uncinella*, in addition to investigating and relating their antioxidant activity to the antitumor potential action of this species in bladder cancer and glioblastoma cells. For *Baccharis* species like, such as *B. trimera* and *B. dracunculifolia*, have reports in the literature of antitumor action. *B. trimera* was tested on cervical cancer cells and showed dose-dependent action for these cells [19]. *B. dracunculifolia*, on the other hand, has an antiproliferative action in a series of tumor cells such as leukemia, prostate cancer, cervical cancer, breast cancer, among others [36]. To our best knowledge, no reports of anti-tumor action were found for extracts of *B. uncinella*, however, the choice of this *Baccharis* species is based on previous studies [37–40], which report the biological activities of its extracts such as sedative, anti-inflammatory, against *Chagas* disease and leishmaniasis, and due to the abundance of this vegetation in southern Brazil. In addition to the anti-tumor evaluation, toxicity against normal cells will also be evaluated.

2. Materials and methods

2.1. Plant material

Aerial parts of *Baccharis uncinella* were collected in January 2017, during the summer in the southern hemisphere, from a native population at the *Pró-Mata Environmental Research and Preservation Center*, *São Francisco de Paula* municipality, state of *Rio Grande do Sul*, southern Brazil. A voucher specimen (MCPUC10437) was deposited at the Herbarium of the Science and Technology Museum of the Pontifical Catholic University of *Rio Grande do Sul* (Herbarium MPUC). After collection, the material was dried in an oven (Tecnal) and ground in a knife mill (Skymsen) for subsequent extraction by supercritical fluid of the non-volatile compounds present in the plant material.

2.2. Extraction and fractionation

The procedure for the extraction of non-volatile compounds by supercritical fluid, or supercritical extraction, is performed using CO_2 as a solvent under two different pressure and one temperature conditions. The pressure and temperature conditions used were chosen in order to maximize the extraction of phenolic compounds based on previous work [39,41,42].

Detailed information of the extraction process and equipment can be found at [43]. A mass of 100 g of plant was added to the extraction vessel and the supercritical extraction occurred at a temperature of 60 °C and a pressure of 150 and 200 bar sequentially, that is, using the same plant material. Ethanol at 10 % by mass of CO_2 was used as a co-solvent. CO_2 has a limited capacity to dissolve polar compounds at high pressures and the use of a co-solvent is justified by the increased solvency and solubility of the supercritical extraction process, consequently increasing the extraction of more polar compounds such as polyphenols [4,14,44].

After extraction, the extract was fractionated by polarity in a glass column, with vacuum to aid elution. A volume of 150 mL of each eluent was added to a 10 cm high silica gel bed (Merck), with a previously dry mass of 0.5 g extract. The eluents used were, in the elution order: hexane, dichloromethane, ethyl acetate and methanol (Merck).

2.3. Polyphenols and antioxidant activity (AA) analysis

The qualitative verification of the polyphenols was carried out through thin layer chromatography (TLC) using as a colored reagent an aqueous solution of potassium ferricyanide (1%) and ferric chloride (2%). Analyzes were performed on Alugram® Xtra SIL G/UV254 silica gel plates (Macherey-Nagel) which were eluted with dichloromethane (Merck). After elution, the plates were sprayed with the polyphenol-colored reagent and the presence of polyphenols is verified through the blue color of the compounds eluted.

The analysis of the AA of the crude supercritical extract and the fractions obtained through fractionation was carried out using the DPPH free radical capture method, proposed by Brand-Williams [45,46]. This method is based on the capture of the DPPH radical (2,2-diphenyl-1-picryl-hydrazil) by antioxidant compounds, producing a decrease in absorbance at 515 nm of wavelength. Quercetin was used as a control at the concentration of 10 mg/mL.

Solutions of 10 mg/mL of sample and ethanol (99.5 % - Merck) and a solution of DPPH (Sigma®) at 60 μM, also in ethanol, were prepared. A 0.1 mL aliquot of the sample solution was placed in glass cuvettes with 3.9 mL of the DPPH solution. The blank was performed by replacing the sample rate with the same volume of ethanol. Quantification was performed using a DPPH calibration curve, with concentrations ranging from 10 μM to 50 μM. The absorbance reading was performed on a Biospectro SP-220 spectrophotometer, at a wavelength of 515 nm and continued until there was no further variation in the absorbance value, around 60 min.

For the fraction that showed the highest AA, a greater concentration range was tested to verify the relationship between AA and toxicity in tumor cells. The same concentrations used in the cell viability test were also tested for AA: 1 at 1000 μg/mL. This fraction was also tested of 1–10 mg/mL to observe the development of the AA curve until reaching the maximum value. The readings were taken after 24 h of preparation, to simulate the incubation period in the cell viability test.

A calculation as a percentage of DPPH free radical scavenging (% FRS) is presented, which indicates the percentage of DPPH consumed. The calculation is shown in Eq. 1:

$$\%FRS = [(\text{abs control} - \text{abs sample}) / (\text{abs control})] \times 100\ \% \quad (1)$$

2.4. Cell viability assay

In order to determinate cytotoxic effect of *B. uncinella* fraction with the highest AA, tumor cell lines including human bladder cancer cells (T24), human glioblastoma cells (U87), and non-tumoral African green monkey kidney cells (Vero) (from American Type Culture Collection) were used. T24 cells were maintained in RPMI cell culture medium, while U87 and Vero cells in DMEM, both supplemented with 10 % fetal bovine serum (FBS), 0.5 U/mL of penicillin and streptomycin. The cells were kept in a humidified cell incubator (37 °C, 5% CO_2, and 95 % humidity) until the experiments, when they were lifted from the sub confluent cultures with 0.5 % trypsin in 5 mM EDTA, counted on a hemocytometer, and plated at the appropriate density according to the experimental protocol.

Cellular viability was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. T24, U87 and Vero cells were plated at a density of 5×10^3 cells per well in 96-well plates. In order to determine toxicity, normal monkey kidney cells (Vero) were exposed to different concentrations of the extract that showed highest AA (10; 25; 50; 75; 100; 200; 250; 500; 750 and 1000 μg/mL) for 24 h. To determine antitumor activity, tumor strains were treated with a smaller range of concentrations (10; 25; 50; 75; 100 and 200 μg/mL) and incubated for 24 h. The concentrations tested in tumor cells were lower than those used for normal cells as they were selected from the results previously presented with Vero cells. Toxic concentrations for normal cells have not been tested for tumor cells as their effect would be more harmful than beneficial. Following treatment, culture medium containing the treatment was removed and the cells were washed twice with PBS (phosphate-buffered saline). Then, 90 μL of culture medium and 10 μL of MTT stock solution (5 mg/mL in PBS) were added to each of the wells. The cells were incubated for 3 h and the solution was then removed from the precipitate. The formazan product in cells was solubilized by adding dimethyl sulfoxide and the level of absorbance was read by an enzyme-linked immunosorbent assay plate reader at 570 nm and 630 nm (absorbance at 570 nm minus absorbance at 630 nm). This absorbance was linearly proportional to the number of live cells with active mitochondria. The results were determined as a percentage of the treated absorbance in relation to the group that did not receive treatment (control).

The statistical test used was a one-way analysis of variance (ANOVA), followed by the Tukey post hoc test. Results were presented as the standard error of the mean. GraphPad Prism 5.0® program was used to generate graphs. P values < 0.05 were taken to indicate statistical significance.The half maximal inhibitory concentration (IC_{50}) was calculated by linear regression.

3. Results

The total yield of extraction and fractionation is show in Table 1.

The sum of the mass recovered in the fractionation (0.35 g) is not the same as the mass inserted in the column (0.5 g). This difference is due to the material that is permanently retained from the silica and to losses during the experiment, such as changing the collection flasks and in the drying process of the fractions.

Table 1
Yields of the crude supercritical extract and fractions obtained from crude supercritical extract.

Sample	Mass (g)	Yield (%w/w)
Extraction results		
Crude extract	4.80	4.80
Fractionation results		
Hexane fraction	0.0955	19.10
Diclorometane fraction	0.0184	3.68
Ethyl acetate fraction	0.1984	39.68
Methanolic fraction	0.0753	15.06

After extract fractionation, all fractions were subjected to qualitative analysis of polyphenols and to evaluation of antioxidant activity as follows. The results can be seen in Figs. 1 and 2 respectively.

As can be seen in Fig. 1, only the hexane fraction did not present amounts of polyphenols in its composition. In the fraction of ethyl acetate and methanol, more polar phenolic compounds are observed that have not been eluted with dichloromethane. The dichloromethane fraction showed phenolic compounds with a greater range of polarity. The relationship between the qualitative analysis of phenolic compounds and the high antioxidant action of the fractions can be seen in the results that follow (Fig. 2).

The methanolic fraction showed the highest AA, when compared to the crude extract and the other fractions, as can be seen at Fig. 2. The percentage of free radical scavenging (% FRS) for this fraction (86.7 %) was very close to the antioxidant standard used in the test, quercetin (87.9 %). The methanolic fraction was used for the tests of cell viability. Phenolic compounds tend to have a strong AA and are normally compounds with high polarity, being representative in the fraction in question.

Fig. 1. Polyphenol qualitative analysis of *Baccharis uncinella* fractions obtained by column chromatography. H-hexane fraction; A-ethyl acetate fraction; D-dichloromethane fraction; M-methanolic fraction.

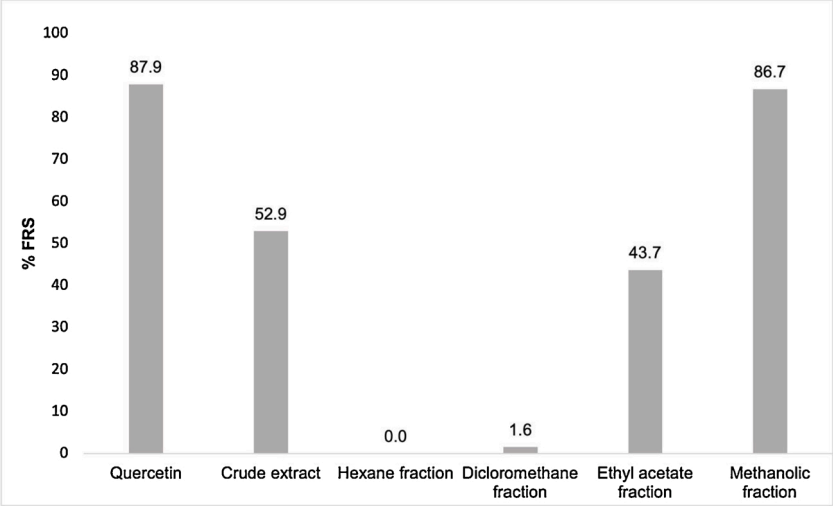

Fig. 2. %FRS of the quercetin standard, crude extract and its fractions.

For the methanolic fraction, in order to make a parallel with the toxicity against tumor cells, the same concentrations tested in the toxicity test were used in the analysis of AA. Fig. 3 shows the evolution of AA, expressed by percentage of free radical scavenging, in the concentrations used in the toxicity tests until reaching its maximum value.

In Fig. 3 can be observed that at very low concentrations (0–50 μg/mL) there is a great variation in the values presented, assuming a more characteristic profile from 100 μg/mL. The AA value slowly increases to a concentration of 250 μL/mL, increasing significantly from 500 μg/mL until reaching its maximum peak at approximately 5000 μg/mL. As it is a colorimetric method and due to the fact that the sample has color, the variations presented by the data may be due to this characteristic of the sample.

Antitumoral effect of *B. uncinella* methanolic fraction was evaluated in different tumor cell lines (T24 and U87) and also in non-tumoral cells (Vero). Cells were exposed to increasing concentrations from 10 to 1000 μg/mL for 24 h and cell viability was measured using MTT assay. Results are shown in Fig. 4. For tumor cells, after 200 μg/mL there was a significant drop in cell viability.

In T24, the control treatment (H_2O) showed a viability of 104.5 ± 3.24 %, while for cells treated with the *B. uncinella* fraction at 200 μg/mL there was a reduction of this value to 15.07 ± 1.10 %, with IC_{50} about 158.8 μg/mL. For U87, the control treatment (H_2O) showed a viability of 100.2 ± 2.69 % and there was a significant reduction at 100 μg/mL, with viability values of 84.87 ± 3.84 % and 50.13 ± 2.15 % for concentrations of 100 and 200 μg/mL, respectively. The IC_{50} for U87 was 213.9 μg/mL.

These values are in agreement with [19] who evaluated the anti-proliferative effect of *B. trimera* extracts for cervical cancer cells (SiHa cells), and who pointed out that IC_{50} concentrations from 400 μg/mL with dose dependent effect are effective in reducing the viability of these cells. Higher IC_{50} values were obtained by Chen et al. [47] against leukemia cells and colon cancer cells for *B. dracunculifolia* supercritical extracts, which demonstrates an effective potential of *B. uncinella* extracts in the development of new chemotherapeutic agents due to lower IC_{50} values found for the cells tested compared to other *Baccharis* species.

The fraction of the supercritical extract of *B. uncinella* showed a better growth inhibitory action for T24 bladder cancer cells, with a lower IC_{50} value than for U87 gliobastoma cells. The values found for the IC_{50} are in accordance with the values found by Konno et al. [48] for mushroom extracts in T24 cells. The authors also show a relationship between the addition of an antioxidant element, the vitamin C, to the mushroom extracts that potentiated the antiproliferative effect in concentrations that, before the combination, were not lethal to the cells. In other studies developed by the authors [49], a less toxic effect of vitamin C was observed for normal cells than for tumor cells. It was observed that this behavior may be related to the pro-oxidant action of vitamin C,

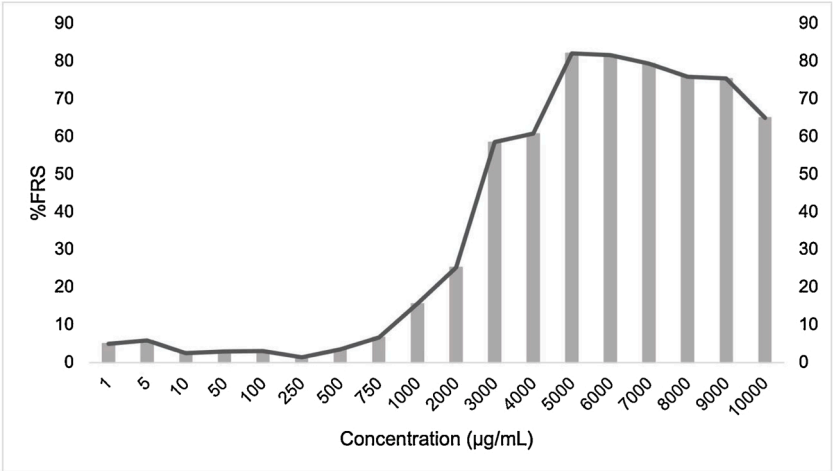

Fig. 3. %FRS evaluation of the methanolic fraction in different concentrations: from 1 to 10,000 μL/mL.

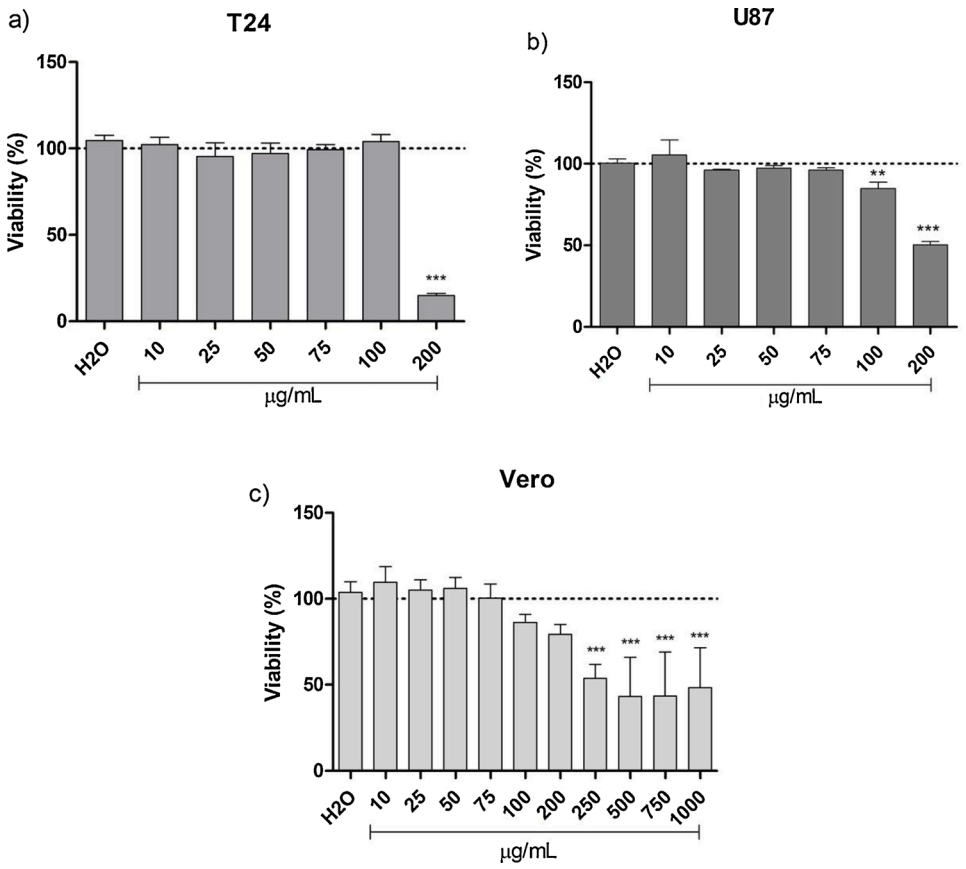

Fig. 4. Effect of *B. uncinella* methanolic fraction on cell viability in tumor cell lines a) T24, b) U87 and c) non-tumoral Vero cells using the MTT assay after 24 h. The data represent the average of four independent experiments carried out in triplicate ± SD. The data were analyzed by One-way ANOVA, followed by Tukey post-hoc. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

which can be extended to studies with *B. uncinella* that have as their main biological activity the high antioxidant activity of their extracts.

Comparing with cisplatin the values are much higher. For T24, the IC_{50} of cisplatin is around 15 µg/mL and 0.3 µg/mL for U87 [24,35]. These differences are expected due to complexity of extracts in relation to pure molecule. This fact can also be observed when a pure natural compound is evaluated for its antiproliferative effect [21,50].

Vero cells were used to investigate the effects of *B. uncinella* extract on non-tumoral cells. As can be observed at Fig. 4.c, the viability value of control (H_2O) was 103.74 ± 6.21 % and a significant antiproliferative effect was perceived at Vero cells from concentration of 250 µg/mL with cell viability value of 53.8 ± 8.05 %. The IC_{50} was 702.5 µg/mL, much higher concentration than used in tumor cells. Making a comparison with cisplatin, IC_{50} concentration for Vero cells is 36 µg/mL [22]. This indicates that the fraction of *B. uncinella* has lower cytotoxic effect on normal cells than on tumor cells, which is important for antineoplastic agents. Selectivity is a major current factor in cancer therapy and natural products presenting this characteristic are favorable candidates to be utilized into new chemotherapeutic agents.

Over last decades, polyphenols received considerable attention for their health benefits, including chemo preventive effects in cancer. These metabolites are considered critical components of plant defense against predation, particularly due their antioxidant capacity [51,52]. *Baccharis* extracts are rich in phenolic compounds, which present great antioxidant activity [39].

In this study, our data available that the cytotoxic effects on tumor cells are related to the concentration of the extract to which AA continuously increases (around 200 µg/mL). The mechanism related to antiproliferative effect of extracts rich in polyphenols has not been clearly defined. Studies suggest that ambiguous relationship between antioxidant and antiproliferative actions may be related to structural characteristics of molecules present in extracts [50]. Most of plant polyphenols retain both antioxidant and prooxidant properties and some studies suggest endogenous cooperation between prooxidant and cytotoxic action of these compounds rather than antioxidant effect in cancer context [51].

4. Conclusion

According to the data analyzed, it can be said that the methanolic fraction of the supercritical extract of *B. uncinella* has an antiproliferative effect for the tumor cells tested, especially for T24 cells, with a less aggressive effect on the normal cells evaluated. For a more effective relationship between the antioxidant action and the antiproliferative effect, more experiments need to be carried out, since the same extract with the same antioxidant activity showed different effects for the cell lines tested, which leads us to believe that the action effect is not the same for all cells.

Author statement

Aline Machado Lucas: Conceptualization; Methodology; Validation; Resources; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing - Review & Editing; Funding acquisition.

Amerícia F. M. L. Bento: Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft.

Rubem Mário Figueiró Vargas: Conceptualization; Validation; Resources; Writing - Original Draft; Writing - Review & Editing.

Thamiris Becker Scheffel: Methodology; Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing -

Review & Editing.

Liliana Rockenbach: Methodology; Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing - Review & Editing.

Fernando Mendonça Diz: Methodology; Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing - Review & Editing.

Angélica Regina Capellari: Methodology; Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft.

Fernanda B. Morrone: Conceptualization; Validation; Resources; Writing - Original Draft; Writing - Review & Editing.

Eduardo Cassel: Conceptualization; Validation; Resources; Writing - Original Draft; Writing - Review & Editing.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

This study was carried out with the financial and technical support of FAPERGS (17/2551-0000884-7) and PUCRS (Edital 01/2017 – Programa de Apoio à Atuação de Professores Horistas em Atividade de Pesquisa). E.C., R.M.F.V., and F.B.M. are recipients of CNPq research fellowship. The authors like to thank all those who made this research possible.

References

[1] L. Gonzaga Verdi, I. Maria Costa Brighente, Moacir Geraldo Pizzolatti, Gênero *Baccharis* (Asteraceae): aspectos químicos, econômicos e biológicos, Quím. Nova 28 (2005) 85–94, https://doi.org/10.1590/S0100-40422005000100017.

[2] J.M. Budel, M.R. Duarte, C.A.M. Santos, P.V. Farago, N.I.O. Matzenbacher, progresso da pesquisa sobre o gênero Baccharis, Asteraceae: I - Estudos botânicos, Rev. Bras. Farmacogn. 15 (2005) 268–271, https://doi.org/10.1590/S0102-695X2005000300018.

[3] S.Q. de Oliveira, G. Barbon, G. Gosmann, S. Bordignon, Differentiation of south Brazilian Baccharis species by TLC, J. Liq. Chromatogr. Relat. Technol. 29 (2006) 2603–2609, https://doi.org/10.1080/10826070600916609.

[4] C.R. Piantino, F.W.B. Aquino, L.A. Follegatti-Romero, F.A. Cabral, Supercritical CO_2 extraction of phenolic compounds from Baccharis dracunculifolia, J. Supercrit. Fluids 47 (2008) 209–214, https://doi.org/10.1016/j.supflu.2008.07.012.

[5] S. dos S. Grecco, L. Gimenes, M.J.P. Ferreira, P. Romoff, O.A. Favero, C. A. Zalewski, J.H.G. Lago, Triterpenoids and phenolic derivatives from B*accharis uncinella* C. DC.(Asteraceae), Biochem. Syst. Ecol. 38 (2010) 1234, https://doi.org/10.1016/j.bse.2010.12.005.

[6] L.F.D. Passero, A. Bonfim-Melo, C.E.P. Corbett, M.D. Laurenti, M.H. Toyama, D. O. De Toyama, P. Romoff, O.A. Fávero, S.S. Dos Grecco, C.A. Zalewsky, Anti-leishmanial effects of purified compounds from aerial parts of *Baccharis uncinella* C. DC.(Asteraceae), Parasitol. Res. 108 (2011) 529–536, https://doi.org/10.1007/s00436-010-2091-8.

[7] B.M. Bocco, G.W. Fernandes, F.B. Lorena, R.M. Cysneiros, M.A. Christoffolete, S. S. Grecco, C.L. Lancellotti, P. Romoff, J.H.G. Lago, A.C. Bianco, M.O. Ribeiro, Combined treatment with caffeic and ferulic acid from *Baccharis uncinella* C. DC. (Asteraceae) protects against metabolic syndrome in mice, Braz. J. Med. Biol. Res. 49 (2016) e5003, https://doi.org/10.1590/1414-431X20155003.

[8] C.H. Baggio, C.S. Freitas, L. Rieck, M.C.A. Marques, Gastroprotective effects of a crude extract of *Baccharis illinita* DC in rats, Pharmacol. Res. 47 (2003) 93–98, https://doi.org/10.1016/S1043-6618(02)00253-0.

[9] S.Q. de Oliveira, F. Dal-Pizzol, G. Gosmann, D. Guillaume, J.C.F. Moreira, E. P. Schenkel, Antioxidant activity of *Baccharis articulata* extracts: isolation of a new compound with antioxidant activity, Free Radic. Res. 37 (2003) 555–559, https://doi.org/10.1080/1071576031000076259.

[10] A.H. Januário, S.L. Santos, S. Marcussi, M.V. Mazzi, R.C.L.R. Pietro, D.N. Sato, J. Ellena, S.V. Sampaio, S.C. França, A.M. Soares, Neo-clerodane diterpenoid, a new metalloprotease snake venom inhibitor from *Baccharis trimera* (Asteraceae): Anti-proteolytic and anti-hemorrhagic properties, Chem. Biol. Interact. 150 (2004) 243–251, https://doi.org/10.1016/j.cbi.2004.09.016.

[11] T. Sartor, V.B. Xavier, M.A. Falcão, C.A. Mondin, M.A. Dos Santos, E. Cassel, L. V. Astarita, E.R. Santarém, Seasonal changes in phenolic compounds and in the biological activities of *Baccharis dentata* (Vell.) GM Barroso, Ind. Crops Prod. 51 (2013) 355–359, https://doi.org/10.1016/j.indcrop.2013.09.018.

[12] S. Boller, C. Soldi, M.C.A. Marques, E.P. Santos, D.A. Cabrini, M.G. Pizzolatti, A. R. Zampronio, M.F. Otuki, Anti-inflammatory effect of crude extract and isolated compounds from *Baccharis illinita* DC in acute skin inflammation, J. Ethnopharmacol. 130 (2010) 262–266, https://doi.org/10.1016/j.jep.2010.05.001.

[13] G.E. Feresin, A. Tapia, A. Gimenez, A. Gutierrez Ravelo, S. Zacchino, M. Sortino, G. Schmeda-Hirschmann, Constituents of the Argentinian medicinal plant *Baccharis grisebachii* and their antimicrobial activity, J. Ethnopharmacol. 89 (2003) 73–80, https://doi.org/10.1016/S0378-8741(03)00259-9.

[14] E. Cassel, R.M.F. Vargas, G.W. Brun, Processos de extração supercrítica aplicados a produtos naturais, in: E. Cassel, L. Machado Rocha (Eds.), Fundam. Tecnol. Prod. Fitoter, EDIPUCRS, Porto Alegre, 2008, pp. 213–228.

[15] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, J. Supercrit. Fluids 38 (2006) 146–166, https://doi.org/10.1016/j.supflu.2006.03.020.

[16] C.H. Collins, G. Braga, P.S. Bonato, Fundamentos de cromatografia, 1st ed., Editora da Unicamp, Campinas, 2006.

[17] C.M. de O. Simões, V. spitzer, Óleos voláteis, in: C.M. de O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. de Mello, L.A. Mentz, P.R. Petrovick (Eds.), Farmacognosia: da Planta ao Medicamento, 6th ed., Editora da UFRGS, Porto Alegre, 2007, pp. 467–485.

[18] M. Sylvestre, A. Pichette, A. Longtin, F. Nagau, J. Legault, Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe, J. Ethnopharmacol. 103 (2006) 99–102, https://doi.org/10.1016/j.jep.2005.07.011.

[19] C.B. De Oliveira, L.N. Comunello, É.S. Maciel, S.R. Giubel, A.N. Bruno, E.C. F. Chiela, G. Lenz, S.C.B. Gnoatto, A. Buffon, G. Gosmann, The inhibitory effects of phenolic and terpenoid compounds from *Baccharis trimera* in Siha cells: differences in their activity and mechanism of action, Molecules 18 (2013) 11022–11032, https://doi.org/10.3390/molecules180911022.

[20] C.B. Pereira, C.C. Kanunfre, P.V. Farago, D.M. Borsato, J.M. Budel, B.H.L. de Noronha Sales Maia, E.A. Campesatto, A. Sartoratto, M.D. Miguel, O.G. Miguel, Cytotoxic mechanism of*Baccharis milleflora* (Less.) DC. essential oil, Toxicol. In Vitro 42 (2017) 214–221, https://doi.org/10.1016/j.tiv.2017.04.031.

[21] L. Alban, Development and Evaluation of Antitumor Activity of Titanate- Modified Nanotubes With Quercetin in Bladder Cancer. Master/PhD Thesis. Graduation Program in Materials Engineering and Technology, Pontifical Catholic University of Rio Grande do Sul, Porto alegre, 2018.

[22] K. Rjiba-Touati, I. Ayed-Boussema, A. Belarbia, A. Azzebi, A. Achour, H. Bacha, Protective effect of recombinant human erythropoeitin against cisplatin cytotoxicity and genotoxicity in cultured Vero cells, Exp. Toxicol. Pathol. 65 (2013) 181–187, https://doi.org/10.1016/j.etp.2011.08.004.

[23] A.I. Elkady, W.S. Ramadan, The aqueous extract of cinnamon bark ameliorated cisplatin-induced cytotoxicity in vero cells without compromising the anticancer efficiency of cisplatin, Biomed. Pap. 160 (2016) 363–371, https://doi.org/10.5507/bp.2016.034.

[24] A. Patricia Moreno-Londoño, C. Bello-Alvarez, J. Pedraza-Chaverri, Isoliquiritigenin pretreatment attenuates cisplatin induced proximal tubular cells (LLC-PK1) death and enhances the toxicity induced by this drug in bladder cancer T24 cell line, Food Chem. Toxicol. 109 (2017) 143–154, https://doi.org/10.1016/j.fct.2017.08.047.

[25] S. Manohar, N. Leung, Cisplatin nephrotoxicity: a review of the literature, J. Nephrol. 31 (2018) 15–25, https://doi.org/10.1007/s40620-017-0392-z.

[26] A.M. Buckley, B.A.S. Bibby, M.R. Dunne, S.A. Kennedy, M.B. Davern, B. N. Kennedy, S.G. Maher, J. O'Sullivan, Characterisation of an isogenic model of cisplatin resistance in oesophageal adenocarcinoma cells, Pharmaceuticals 12 (2019) 1–20, https://doi.org/10.3390/ph12010033.

[27] D. Bagchi, A. Swaroop, H.G. Preuss, M. Bagchi, Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview, Mutat. Res. - Fundam. Mol. Mech. Mutagen. 768 (2014) 69–73, https://doi.org/10.1016/j.mrfmmm.2014.04.004.

[28] A.M. Kamat, N.M. Hahn, J.A. Efstathiou, S.P. Lerner, P.U. Malmström, W. Choi, C. C. Guo, Y. Lotan, W. Kassouf, Bladder cancer, Lancet 388 (2016) 2796–2810, https://doi.org/10.1016/S0140-6736(16)30512-8.

[29] A. Matsos, I.N. Johnston, Chemotherapy-induced cognitive impairments: a systematic review of the animal literature, Neurosci. Biobehav. Rev. 102 (2019) 382–399, https://doi.org/10.1016/j.neubiorev.2019.05.001.

[30] K. Anjum, B.I. Shagufta, S.Q. Abbas, S. Patel, I. Khan, S.A.A. Shah, N. Akhter, S. S. ul Hassan, Current status and future therapeutic perspectives of glioblastoma multiforme (GBM) therapy: a review, Biomed. Pharmacother. 92 (2017) 681–689, https://doi.org/10.1016/j.biopha.2017.05.125.

[31] M.P. Gehring, F. Kipper, N.F. Nicoletti, N.D. Sperotto, R. Zanin, A.S.K. Tamajusuku, D.G. Flores, L. Meurer, R. Roesler, A.B. Filho, G. Lenz, M.M. Campos, F.B. Morrone, P2X7 receptor as predictor gene for glioma radiosensitivity and median survival, Int. J. Biochem. Cell Biol. 68 (2015) 92–100, https://doi.org/10.1016/j.biocel.2015.09.001.

[32] S. Dasari, P. Bernard Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, Eur. J. Pharmacol. 740 (2014) 364–378, https://doi.org/10.1016/j.ejphar.2014.07.025.

[33] R.K. Mehmood, Review of cisplatin and oxaliplatin in current immunogenic and monoclonal antibodies perspective, Oncol. Rev. 8 (2014) 36–43, https://doi.org/10.4081/oncol.2014.256.

[34] S.A. Aldossary, Review on pharmacology of cisplatin: clinical use, toxicity and mechanism of resistance of cisplatin, Biomed. Pharmacol. J. 12 (2019) 7–15, https://doi.org/10.13005/bpj/1608.

[35] H.I. Kim, S.H. Hong, J.M. Ku, M.J. Kim, S.W. Ju, S.W. Chang, C. Cheon, S. Ko, Apoptosis of glioblastoma cells via AKT / mTOR pathway while protecting death of astrocytes, Nutrients 12 (2020) 196, https://doi.org/10.3390/nu12010196.

[36] M. Shahinozzaman, B. Basak, R. Emran, P. Rozario, D.N. Obanda, C. Artepillin, A comprehensive review of its chemistry, bioavailability, and pharmacological

properties, Fitoterapia 147 (2020) 104775, https://doi.org/10.1016/j.fitote.2020.104775.
[37] C.A. Zalewski, L.F.D. Passero, A.S.R.B. Melo, C.E.P. Corbett, M.D. Laurenti, M. H. Toyama, D.O. Toyama, P. Romoff, O.A. Fávero, J.H.G. Lago, Evaluation of anti-inflammatory activity of derivatives from aerial parts of *Baccharis uncinella*, Pharm. Biol. 49 (2011) 602–607, https://doi.org/10.3109/13880209.2010.537828.
[38] J. Ascari, S.L. Sens, D.S. Nunes, A. Wisniewski, M.D. Arbo, V.M. Linck, P. Lunardi, M.B. Leal, E. Elisabetsky, Sedative effects of essential oils obtained from *Baccharis uncinella*, Pharm. Biol. 50 (2012) 113–119, https://doi.org/10.3109/13880209.2011.634423.
[39] A.M. Lucas, Systematic Study for the Supercritical Obtention and Impregnation of *Baccharis*. PhD Thesis. Graduation Program in Materials Engineering and Technology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, 2015.
[40] R.N.M. Neto, R.F.B. Setúbal, T.M.M.I. Higino, M.C.A. Brelaz-De-Castro, L.C.N. Da Silva, A.S. Dos Santos Aliança, Asteraceae plants as sources of compounds against leishmaniasis and chagas disease, Front. Pharmacol. 10 (2019) 1–20, https://doi.org/10.3389/fphar.2019.00477.
[41] E. Cassel, R.M.F. Vargas, G.W. Brun, D.E. Almeida, L. Cogoi, G. Ferraro, R. Filip, Supercritical fluid extraction of alkaloids from *Ilex paraguariensis* St. Hil, J. Food Eng. 100 (2010) 656–661, https://doi.org/10.1016/j.jfoodeng.2010.05.015.
[42] M.S.T. Barroso, G. Villanueva, A.M. Lucas, G.P. Perez, R.M.F. Vargas, G.W. Brun, E. Cassel, Supercritical fluid extraction of volatile and non-volatile compounds from *Schinus molle* L, Braz. J. Chem. Eng. 28 (2011) 305–312, https://doi.org/10.1590/S0104-66322011000200015.
[43] R. Scopel, R.G. Neto, M.A. Falcão, E. Cassel, R.M.F. Vargas, Supercritical CO_2 extraction of *Schinus molle* L. with co-solvents: mathematical modeling and antimicrobial applications, Braz. Arch. Biol. Technol. 56 (2013) 513–519, https://doi.org/10.1590/S1516-89132013000300020.
[44] R.N. Almeida, R.G. Neto, F.M.C. Barros, E. Cassel, G.L. von Poser, R.M.F. Vargas, Supercritical extraction of *Hypericum caprifoliatum* using carbon dioxide and ethanol+water as co-solvent, Chem. Eng. Process. Process Intensif. 70 (2013) 95–102, https://doi.org/10.1016/j.cep.2013.05.002.
[45] W. Brand-Williams, M.-E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT-Food Sci. Technol. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.
[46] M. Rufino, R.E. Alves, E.S. de Brito, S.M. de Morais, C. de G. Sampaio, J. Pérez-Jimenez, F.D. Saura-Calixto, Metodologia científica: determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH, Embrapa Agroindústria Trop. Comunicado Técnico 127 (2007).
[47] C.R. Chen, Y.N. Lee, M.R. Lee, C.M.J. Chang, Supercritical fluids extraction of cinnamic acid derivatives from Brazilian propolis and the effect on growth inhibition of colon cancer cells, J. Taiwan Inst. Chem. Eng. 40 (2009) 130–135, https://doi.org/10.1016/j.jtice.2008.07.014.
[48] S. Konno, Effect of various natural products on growth of bladder cancer cells: two promising mushroom extracts, Altern. Med. Rev. 12 (2007) 63–68.
[49] Q. Chen, M.G. Espey, M.C. Krishna, J.B. Mitchell, C.P. Corpe, G.R. Buettner, E. Shacter, M. Levine, Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13604–13609, https://doi.org/10.1073/pnas.0506390102.
[50] I. Grigalius, V. Petrikaite, Relationship between antioxidant and anticancer activity of trihydroxyflavones, Molecules 22 (2017), https://doi.org/10.3390/molecules22122169.
[51] H. Yar Khan, H. Zubair, M. Fahad Ullah, A. Ahmad, S. Mumtaz Hadi, A prooxidant mechanism for the anticancer and chemopreventive properties of plant polyphenols, Curr. Drug Targets 13 (2012) 1738–1749, https://doi.org/10.2174/138945012804545560.
[52] H.O. Elansary, A. Szopa, P. Kubica, F.A. Al-Mana, E.A. Mahmoud, T.K. Ali, Z. El-Abedin, M.A. Mattar, H. Ekiert, Molecules phenolic compounds of *Catalpa speciosa*, *Taxus cuspidata*, and *Magnolia acuminata* have antioxidant and anticancer activity, Molecules 24 (2019) 412, https://doi.org/10.3390/molecules24030412.