Phytomedicine Plus 2 (2022) 100273

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Caryocar brasiliense Camb. fruit peel butanolic fraction induces antiproliferative effects against murine melanoma cell line

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ARTICLE INFO

Keywords:
Medicinal plant
Cutaneous malignant melanoma
Phenolic compounds
Folk medicine cells
Chromatography

ABSTRACT

Background: Cutaneous malignant melanoma is a skin cancer type highly resistant to standard cancer therapies. Natural compounds have been reported as important sources for the creation of new drugs for cancer treatment. *Caryocar brasiliense* Camb., popularly known as Pequi, is often used in Brazilian folk medicine, with anticancer effects reported. Objectives: The present study evaluated the antiproliferative activity of the crude extract butanolic fraction of the *C. brasiliense* Camb. peel on the B16F10 cell linage

Method: C. brasiliense peel fraction was analyzed by Gas Chromatography coupled to massa spectra, and its biological effects were evaluated on the melanoma cell line B16F10.

Results: The chromatography analysis of the butanolic fraction of the *C. brasiliense* peel fraction identified a majority presence of gallic acid and sarothrin compounds. These compounds might have been responsible for an antiproliferative effect on B16F10, with the inhibitory concentration (IC_{50}) equal to 390.9 μg/mL (24 h) and 226.4 μg/mL (48 h) after treatments. Our results revealed that cell death assay via bromide and acridine orange tests indicated an increase in cell death observed after 24 h treatment with the pequi fraction at 250 μg/mL ($p < 0.05$) and 500 μg/mL ($p < 0.01$). In addition, a significant increase in cell death at 250 μg/mL ($p < 0.01$) and 500 μg/mL ($p < 0.0001$) occurred after 48 h. Furthermore, a significant reduction in migratory activity in cells treated at 250 μg/mL ($p < 0.05$) and 500 μg/mL ($p < 0.01$) occurred and was enhanced by the 48 h treatment ($p < 0.001$).

Conclusions: The present study is the first to demonstrate the use of "Pequi" residual by product as a potential reservoir of bioactive compounds with antiproliferative activity on B16F10 melanoma cells.

Introduction

Malignant melanoma is considered a public health problem responsible for more than 90% of deaths from skin cancer (Burns et al., 2019). Cutaneous malignant melanoma (CMM) is associated with risk factors such as racial skin phenotype, family history, erratic genetic factors, and UV light exposure (Michielin et al., 2019), which CMM affecting mainly young and middle-aged populations (Coricovac et al., 2018; Leonardi et al., 2018). Cutaneous melanoma has its genesis in epidermal melanocytes and is characterized as a highly metastatic neoplasm (Schadendorf et al., 2018; Schatton et al., 2008). Although CMM represents 5% of cutaneous malignancies, it accounts for high mortality and

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https://doi.org/10.1016/j.phyplu.2022.100273

Available online 4 April 2022

morbidity rates, in addition to being resistant to conventional therapies, such as chemotherapy (Uscanga-Palomeque et al., 2019).

Given the therapeutic challenges of CMM, new compounds derived from natural products have emerged in the search for novel drugs with relevant pharmacological properties, such as cytotoxicity and chemotherapy activity against cancer (Figueiredo et al., 2014). In this regard, Brazil is a rich source of flora biodiversity, including in the Brazilian Savannah, also known as the Cerrado, widely distributed across the central plateau. *Caryocar brasiliense* Camb., a native species popularly known as pequi (Colombo et al., 2015), is of interest due to its high levels of several natural antioxidants, such as gallic acid, quinic acid, quercetin, and quercetin 3-*O*-arabinose (Breda et al., 2016; Roesler et al., 2008). In folk medicine, pequi is known for its anti-inflammatory, tonic, and aphrodisiac properties (de Oliveira et al., 2018). The oil from its pulp is commonly used to treat bronchitis, colds, and flu and to control tumors (Roll et al., 2018). Furthermore, the antitumor activity of the *C. brasiliensis* fruit has been proven and is related to its antioxidant property (Colombo et al., 2015). Studies have also demonstrated chemopreventive effects of pequi oil on preneoplastic lesions in a mouse hepatocarcinogenesis model (Colombo et al., 2015; Palmeira et al., 2016).

Considering the potential of pequi, this study investigated the antineoplastic activity of the butanolic fraction of the fruit peel of *C. brasiliense* Camb. on the CMM B16F10 murine cell line. The B16 cell line is the standard for conducting melanoma research because of its rapid and aggressive growth. *In vitro* studies occur before *in vivo* tests to optimize therapeutic efficacy. Therefore, the use of the B16F10 melanoma strain, one of the few pigmented melanoma strains that can be used in studies with mice, is ideal for carrying out preliminary tests and for later application in an *in vivo* model in mice (Nakamura et al., 2002; Overwijk and Restifo, 2000; Zaidi et al., 2008). The murine model is widely used in biomedical research for conserving almost 99% of human genes and is physiologically similar to humans (Dutta and Sengupta, 2016).

Materials and methods

Plant material

"Pequi" fruits were purchased at the market in the city of Montes Claros, Minas Gerais. Geraldo Melo PhD identified the species, and the plant name was checked using the www.theplantlist.org website. The criterion for fruit selection was the healthy appearance of the fruit peel (external mesocarp and exocarp). Fruits were then cut and dehydrated in an oven with forced air circulation (model 400/ND/Nova Ética) at a temperature of 45 °C. After drying, the peels were crushed to a fine powder in a mechanical mill, followed by conditioning at room temperature until use. The powdered material was protected from light to prevent photodegradation.

Extract and fraction

The extraction process was performed according to Sidônio (2009). The previously pulverized material was mixed with 80% methanol as an extraction solvent, in the proportion of 50 g of material to 300 mL of solvent. After 24 h of extraction under periodic stirring, the mixture was filtered, and the filtrate, considered to be the crude hydromethanolic extract, was obtained. The extract was placed in an oven with forced air circulation (model 400/ND/Nova Ética) at 45 °C to reduce its initial volume.

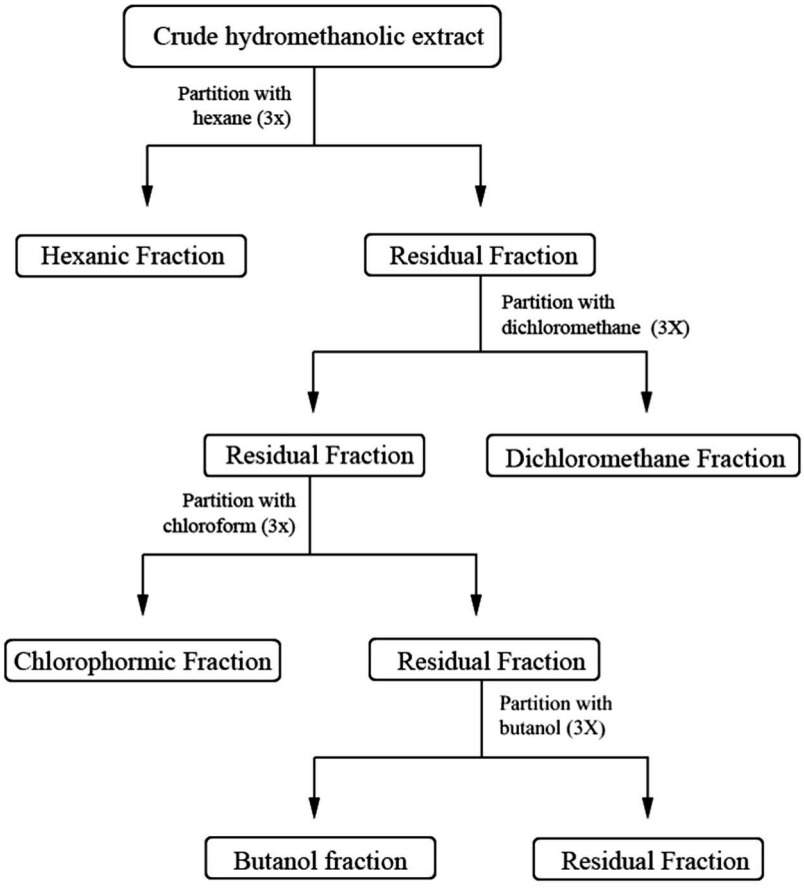

Fig. 1. Flowchart of the butanolic fractionation of the crude extract of the fruit peel (*Caryocar brasiliense* Camb.) by the liquid-liquid partition technique, using the solvents hexane, dichloromethane, chloroform and butanol, separating classes of compounds according to the polarity increment.

For liquid-liquid fractionation procedures, distilled water was added to the crude hydromethanol extract, in the proportion of 50 mL of water to 100 mL of extract (Fig. 1). This procedure was performed to provide better partitioning during fractionation as per previous laboratory optimization protocols. The fractionation was performed using butanol followed by drying at 45 °C. The resulting fraction was then stored in a freezer in a hermetic glass until use *in vitro* tests.

GC–MS analysis

Aliquots 2 mg of the plant extracts were measured in an internally conical glass suitable for this process and then dissolved in 60 μL of pyridine and 100 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide containing 1% of chlorotrimethylsilane. The reaction mixture was heated at 60 °C for 30 min. A volume of 1 μL from the sample was injected into the GC–MS column.

The chromatographic analyses were performed on an gas chromatograph (GC 7890A, Agilent Technologies) equipped with an electron impact ionization detector (GC–MS) and a DB-5MS capillary column (Agilent Technologies, 30 m length x 0.25 mm internal diameter x 0.25 μm film thickness). Helium (99.9999% purity) was used as carrier gas at a rate of 1 mL min^{-1}. Using an autoinjector (CTC combiPaL), 1 μL of the sample was injected into the chromatograph at a 1:10 split ratio. The split/splitless injector was kept at 230 °C. The chromatographic column was heated at a rate of 10 °C min^{-1} from 80 up to 300 °C with an isotherm of 38 min. The interface temperature was maintained at 280 °C and the ionization was performed by the impact of 70 eV, with the ion source at 230 °C. The *m/z* sweep range was from 50 to 650 Da. Identification of compounds was performed based on the mass comparison method using as reference the NIST library and the PubChem platform (https://pubchem.ncbi.nlm.nih.gov/).

Cell culture

In this study, we used murine metastatic melanoma cells of the B16F10 line provided by the Federal University of Minas Gerais - UFMG. For the cultivation of B16F10 cells, RPMI medium was used (Gibco™ RPMI Medium 199 Powder, 10 × 1 L), supplemented with 10% inactivated fetal bovine serum (Gibco™ fetal bovine serum, certified, heat-inactivated, US) and 1% antibiotic (Penicillin/Streptomycin). Cells were maintained in tissue culture bottles in an incubator at 37 °C in a humidified atmosphere with 5% CO_2 and observed daily (Sterisonic™ GxP MCO-19AIC (UVH) cell incubator CO_2 culture. SANYO Electric Co. Ltd.).

Extract preparation and treatments

To prepare the extracts, 50 mg of the butanolic fraction of the crude extract of the fruit peel of *Caryocar brasiliense* Camb. was added to 5 mL of 1x PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4). After shaking, the mixture was filtered with a syringe through a 0.22 μm Milli pore filter. B16F10 cells (2.5×10^3 cells/well) were seeded in polystyrene microplates (96 wells) for 24 h and subjected to treatments with different extract concentrations (1000, 500, 250, and 125 μg/mL) for 24 and 48 h. Cells treated with culture medium and PBS were defined as the control group.

Cell viability

Cell viability was determined by the MTT assay (3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide) adapted from (Riss et al., 2016), as described above. The medium containing the extract in different concentrations was removed and then 150 μL of MTT solution (15 μl of MTT (5 mg/mL) in 135 μL of medium) was added. The mixture was incubated at 37 °C with 5% CO_2 for 2 h and 30 min The medium with MTT was removed and the resulting precipitate was dissolved in 200 μL of dimethylsulfoxide (DMSO) per well, with subsequent shaking under dark condition. The absorbance was then measured at 540 nm using a spectrophotometer (Elisa Microplate Reader/POLARIS). Absorbance was used as an index of cell viability and results were expressed as percentages relative to the PBS-treated control group. For greater reliability, the tests were performed in triplicate and three independent experiments were conducted.

Wound scratch assay

Cell migration was monitored in a wound risk assay as described above (Guimaraes et al., 2016; Guimarães et al., 2016). A density of 7×10^5 cells/well was plated. Treatments were applied after the cells reached a confluent monolayer at 80% of the six-well plate. After 24 and 48 h of treatment, treatments were removed and 200 μL of RPMI medium was added. Cells were immediately observed in an inverted IX81 microscope (Olympus, Center Valley, PA, USA). Slides were photographed using an SC30 camera (Olympus, Center Valley, PA, USA). ImageJ software (NIH, United States) was used to analyze the wound healing area by dividing the initial area by the final cell-free area (Vaupel and Harrison, 2004). Two independent experiments were performed in duplicate.

Cell death assay

B16F10 cells were plated at a density of 7×10^5 cells/well. After 24 and 48 h, treatments were removed from each well and added to a mixture of 100 μL/mL of acridine orange 100 μg/mL (AO, Sigma, St. Louis, MO, USA) and 100 μg/mL of ethidium bromide (EB, Sigma, St. Louis, MO, USA) (1:1). The cells were immediately analyzed using a FSX100 fluorescence microscope (Olympus, Center Valley, PA, USA). Three fields per well were captured at 4X magnification. Viable cells had fluorescent green color shown by intense AO staining (Ex460–495, Em510–550, DM505 filter), and dead cells had bright orange color indicated by intense EB staining (Ex360–370, Em420–460, DM400 filter). The images obtained were analyzed using the ImageJ software (NIH, United States) to quantify the percentage of dead/apoptotic cells (total number of dead cells/total number of cells counted] x 100) (da Rocha et al., 2019).

Data analysis

Statistical analysis of data was performed using the GraphPad Prism software (version 6.0®, San Diego, California, USA), with 95% confidence level ($p < 0.05$). Data are presented as mean ± standard deviation (SD). Statistical differences were assessed through one-way ANOVA was used, followed by the Bonferroni post-test.

Results and discussion

Major compounds of the butanolic fraction of C. brasiliensis Camb

The GC–MS analysis of the pequi peel butanolic fraction revealed the presence of 11 compounds (Fig. 2, Table S1, and Supplementary Figs. S1–S11), which presented a high proportion of sugars. The major constituents were shikimic acid (37.73%), gallic acid (GA; 5.18%), *myo*-inositol (3.79%), and sarothrin (3,6,8-Trimethoxy-4′,5,7-trihydroxyflavone; 2.55%). Among the compounds, we highlight the presence of GA, which was confirmed by comparing its spectroscopic data to those in the NIST library and standard, with a retention time of 14.614 min and a concentration of 41.67 mg/L (Fig. 2B).

The main compounds identified in the butanolic fraction were phenolic. GA, one of the main phenolic compounds identified in the present study, has already been described as the main compound present in the pequi peel ethanolic extract (Nascimento-Silva and Naves, 2019; Rocha et al., 2015). Shikimic acid is an important precursor in the

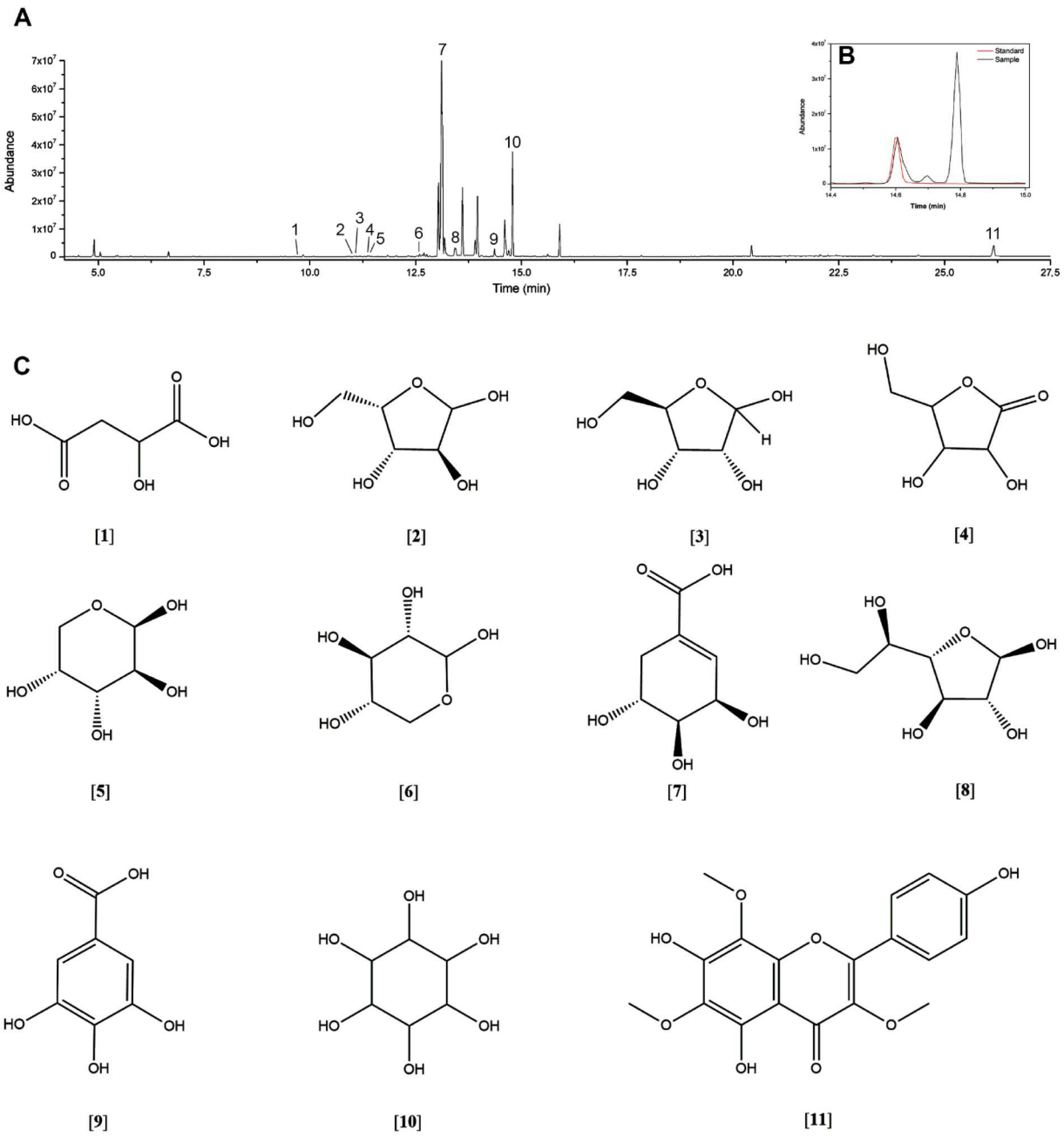

Fig. 2. Profile GC–MS and molecular structure of chemical constituents of butanolic fraction of the crude extract of the peel of *C. brasiliense* Camb. detected by CG-MS. (A) GC–MS profile; (B) Gallic acid standard quantification compared with sample (retention time of 14.614 min with a concentration of 41.67 mg L^{-1}); (C) Molecular structure of the identified compounds. [1] malic acid, [2] D-xylofuranose, [3] D-ribofuranose, [4] arabinonic acid, [5] β-arabinopyranose, [6] d-(+)-xylose, [7] shikimic acid, [8] β-D-galactofuranose, [9] gallic acid, [10] *myo*-inositol, [11] Sarotrin.

synthetic route of phenolic compounds, including GA (Diep et al., 2020). These compounds have antioxidant properties demonstrated by their effective scavenging activity on reactive oxygen species and free radicals and important pharmacological properties, such as antiallergic, anti-inflammatory, antioxidant, antimicrobial, and antitumorigenic properties (Lyu et al., 2020; Nagayoshi et al., 2019).

The pequi fruit peel corresponds to approximately 76% of the fruit (Vera et al., 2005). This by-product is usually discarded and ends up causing discomfort to the population due to deterioration and rancidity in view of the high content of lipids found in the fruit, in addition to promoting contamination of soil and water resources, accompanied by the proliferation of vectors that threaten human health (Siqueira et al., 2012). This ends up requiring significant investments for the treatment of pollution (Abud and Narain, 2009). However, the presence of these compounds in the pequi fruit peel unveils this product as an important source of bioactive compounds, motivating new research for its use in the diet, as well as extracts and isolated compounds.

A butanolic fraction of C. brasiliensis Camb. reduces the viability of B16F10 cells

The *C. brasiliense* Camb. peel butanolic fraction effect on viability is summarized in Fig. 3. Cytotoxicity is an initial indication of the anti-neoplastic activity present in most chemotherapeutic and antitumor agents (Ajith and Janardhanan, 2003). Cell viability assay by the MTT test demonstrated a reduction in the B16F10 cells' viability at doses of

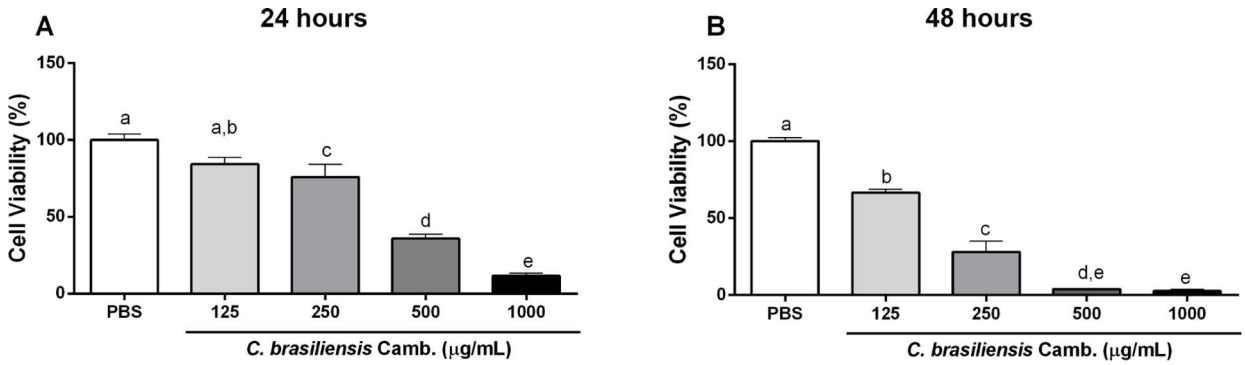

Fig. 3. Effect of the butanolic fraction of the crude extract of the peel of *C. brasiliense* Camb. on cell viability after 24 h (A) and 48 h (B) culture. The B16F10 cells were treated with the extract at various concentrations for 24 and 48 h using MTT assay. The results represent the mean ± SD of three independent experiments, and were analyzed by one-way ANOVA test, Bonferroni post-test. Statistical differences ($p < 0.05$) are shown for groups with different letters (a-e) indicated above the bars of each treatment.

250 ($p < 0.01$), 500, and 1000 µg/mL of the butanolic fraction ($p < 0.0001$) in the 24 h exposure period when compared with the effect of PBS on the control (Fig. 3A). These effects were intensified in the 48 h treatment, with a significant reduction in viability induced by all extract doses ($p < 0.0001$; Fig. 3B). Our results revealed a dose-dependent reduction in cell proliferation, with a 50% inhibitory concentration equal to 390.9 µg/mL and 226.4 µg/mL induced by the 24 h and 48 h treatments, respectively (Fig. 4). Therefore, doses applied in the subsequent trials were chosen based on the described results. A study carried out by Roesler et al., evaluating the cytotoxic effect of pequi peel on 3T3 fibroblast cells, found a low toxicity, with an IC_{50} of 3731.3 µg/mL (Roesler et al., 2010). This data indicates that the biological effects observed in the present assay are attributed to the compounds present in the pequi peel as an anticancer property, considering that the reduction in cell viability by the fraction was caused at doses below 500 µg/mL. However, the mechanisms involved in the process need further investigation to verify if the pequi peel fraction promotes death by cytotoxicity or antiproliferative effect, still both effects. In addition, an analysis on the selectivity of the fraction must be verified in normal cells.

The cell death assay via the bromide and acridine orange tests revealed an increase in cell death observed after treatment with pequi peel butanolic fraction at 250 µg/mL ($p < 0.05$) and 500 µg/mL ($p < 0.01$) when compared with the PBS effect on the control group (Fig. 5A). Similar results were observed after 48 h of treatment, with a marked increase in cell death in the cell group treated with the fraction at 250 µg/mL ($p < 0.01$) and 500 µg/mL ($p < 0.0001$), resulting in a lower cell confluence (Fig. 5B).

The antiproliferative activity of the butanolic fraction can be attributed to the presence of phenolic compounds (Mustapha et al., 2015). Among the major compounds, GA inhibits cell proliferation and induces apoptosis in several cancer cell lines (Lo et al., 2010). GA at 400 µM induces death by apoptosis in B16F10 cells by the mitochondrial pathway through overexpression of cleaved forms of caspase-9, caspase-3 and PARP-1, and pro-apoptotic Bax and Bad, accompanied by Bcl-2 and underexpressed anti-apoptotic Bcl-XL (Liu et al., 2014).

The anti-melanogenic effect of GA is also mediated by pigmentation modulation and adaptation to oxidative stress mediated by melanogenesis. GA regulates the glutathione-cysteine glutamate and glutathione S-transferase redox system at transcriptional and post-translational levels in B16F10 cells (Panich et al., 2012). Furthermore, GA inhibits melanin synthesis and tyrosinase activity and decreases the expression of transcription factors associated with microphthalmia, tyrosinase, tyrosinase-related protein 1, and dopachrome tautomerase, which are also proteins related to melanogenesis. Additionally, GA

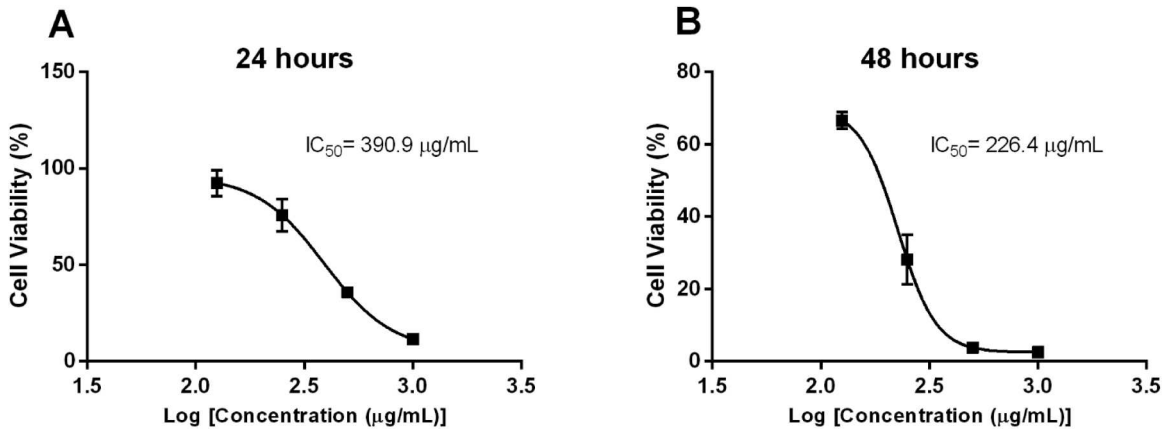

Fig. 4. Inhibitory concentration 50% of the cell viability (B16F10 cells) of butanolic fraction of the crude extract of the peel of *C. brasiliense* Camb. after 24 (A) and 48 (B) h of treatment. The IC50 was calculated by non-linear regression of the curve.

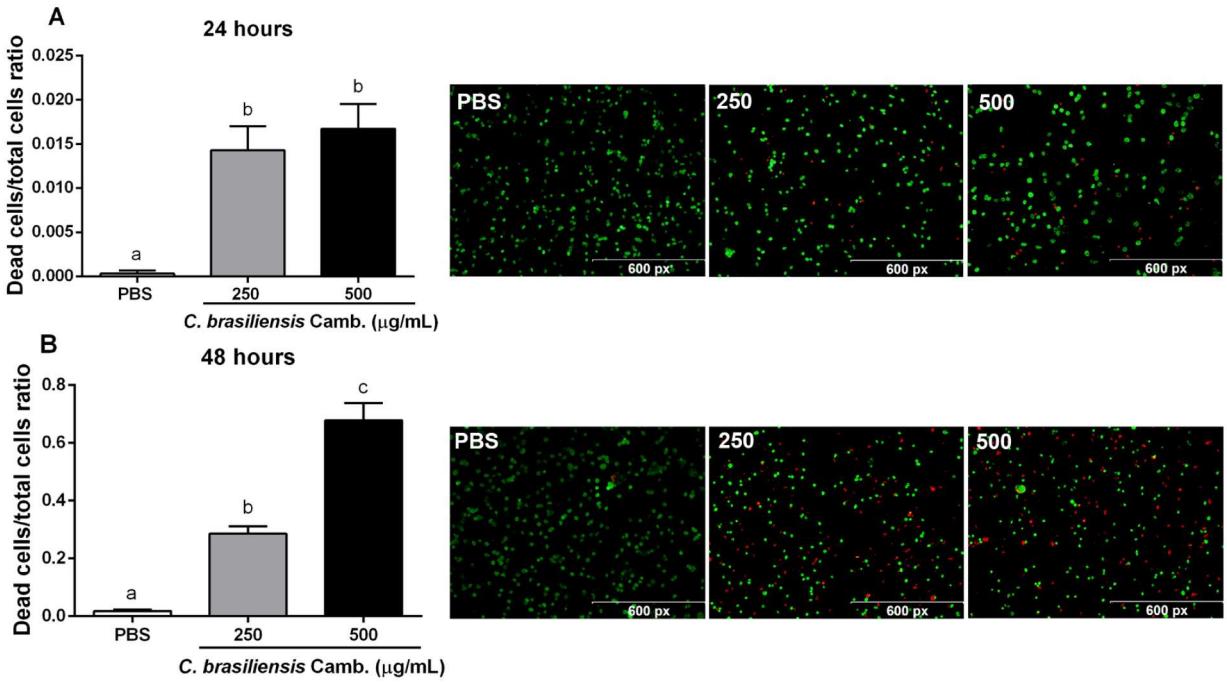

Fig. 5. Butanolic fraction of the crude extract of the peel of *C. brasiliense* Camb. induces death of B16F10 cells. The cancer cells exposed to the butanolic fraction of the crude extract of the peel of C. brasiliense Camb. at concentrations 250 and 500 µg/ml for 24 h (A) and 48 h (B). The results represent the mean ± SD of two independent experiments, and were analyzed by one-way ANOVA test, Bonferroni post-test. Statistical differences ($p < 0.05$) are shown for groups with different letters (a-c) indicated above the bars of each treatment.

phosphorylates and activates melanogenesis-inhibiting proteins, such as Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase (Su et al., 2013). Sarothrin has cytotoxic activity on mouse B16 melanoma cells, as confirmed by an MTT cell viability assay after 48 h of exposure (National Center for Biotechnology Information, 2021). Flavones with a chemical structure similar to that of sarothrin, 2′,4′,6-trimethoxyflavone, 3′,4′,5-trihydroxy-6,7,8-trimethoxyflavone, 2′4′-dihydroxy-6′-methoxy-chalcone, and 4′,5-dihydroxy-6,7,8-trimethoxyflavone have been isolated from Loranthus acutifolius (Apaza Ticona et al., 2020). These compounds have cytotoxic potential against B16F10 cells, which is in accordance with the anti-melanogenic potential observed by the flavone identified in the *C. brasiliensis* Camb. peel butanolic fraction.

Myo-inositol, a sugar alcohol and glucose isomer, is the precursor of numerous secondary messengers. It has a chemopreventive effect on a tobacco-exposed mouse model of lung cancer (Unver et al., 2018). *Myo*-inositol inhibits (phosphatidyl-inositol 3-kinase (PI3K) *in vitro* expression. PI3-kinase is important in the regulation of mitogenesis and cell differentiation (Carvalheira et al., 2002), which are plausible mechanisms of action for the chemopreventive effects (Unver et al., 2018).

The butanolic fraction of C. brasiliensis Camb. reduces migration of B16F10 cells

The cell migration assay revealed that treatment with the pequi peel butanolic fraction reduced cell migration in a concentration and time-dependent manner, indicating a reduced migratory capacity compared to untreated cells (Fig. 6). We observed a significant reduction in migratory activity in cells treated with the butanolic fraction at 250 µg/mL ($p < 0.05$) and 500 µg/mL ($p < 0.01$), and this reduction was enhanced by the 48 h treatment ($p < 0.001$). Notably, we also verified the reduction of cells adhered to the plate, which corroborates findings regarding the reduction of cell viability and death. Furthermore, the 48 h treatment not only reduced the cell count but also increased the wound area, which justifies the negative values found. The inhibitory effect of B16F10 cell migration can be attributed to the presence of GA in the butanolic fraction of *C. brasiliensis*. Evidence has demonstrated that GA can suppress the migration and invasion of human melanoma cells (A375.S2 cells) via the Ras, AKT, and p38 ERK1/2 pathways (Lo et al., 2011) and suppress the migration of B16F10 cells in a dose-dependent manner at different concentrations (50, 200, and 400 µM) and time periods (6, 12, and 24 h; (Lo et al., 2010). For the doses used in our study, 76.1 µM AG was found for the treatment with 250 µg/mL butanolic fraction and 152.2 µM AG for the 500 µg/mL dose of fraction. These results demonstrate an inhibitory effect on B16F10 cells at a lower dose than that found by Lo et al. This can be explained by the synergism of other compounds found in our study already reported in the literature with anticancer activity (Table 1).

Final considerations

The present study is the first to demonstrate the antiproliferative effect of *C. brasiliense* Camb. butanolic fraction peel in the murine melanoma cell line B16F10. The pequi fraction decreased the viability of

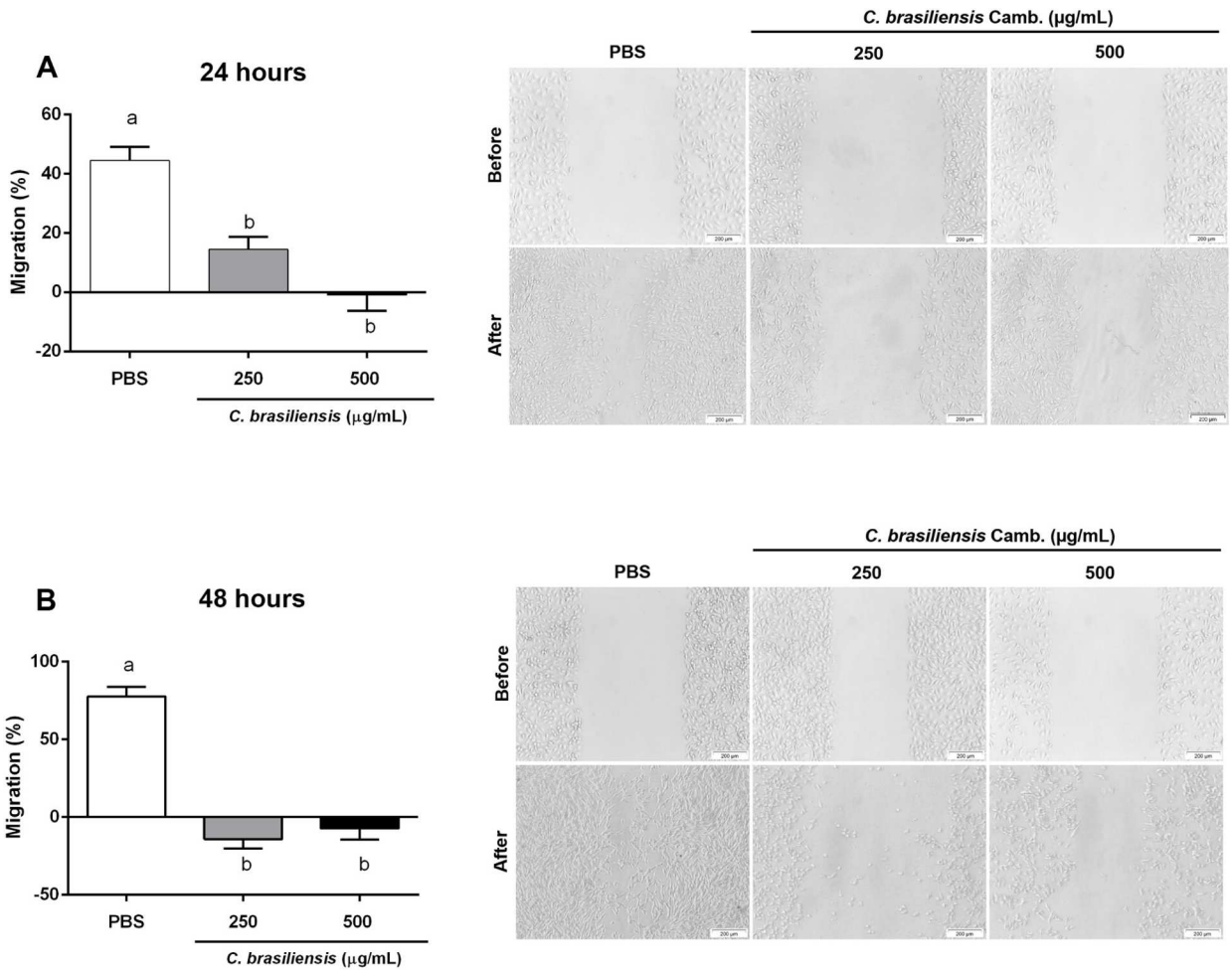

Fig. 6. Effect of butanolic fraction of the crude extract of the peel of *C. brasiliense* Camb. on B16F10 cells migration. Percentage of B16F10 cell migration treated with crude extract of the peel of *C. brasiliense* Camb at the doses of 250 and 500 μg/mL after 24 (A) and 48 h (B) of treatment, when compared to control (PBS). The results represent the mean ± SD of two independent experiments, and were analyzed by one-way ANOVA test, Bonferroni post-test. Statistical differences ($p < 0.05$) are shown for groups with different letters (a and b) indicated above the bars of each treatment.

Table 1
Chemical composition of butanolic fraction of the rind of the fruit of Caryocar Brasiliense Camb.

Peak	Compound	RT	Area	Relative area (%)	Peaks	PubChem CID
1	Malic acid	9.405	2,329,957	0.04	73, 147, 233	522,155
2	D-Xylofuranose	10.880	3,028,605	0.06	217, 73, 147	6,427,436
3	D-Ribofuranose	10.947	2,932,956	0.06	217, 73, 147	13,981,777
4	Arabinonic acid, 1,4-lactone	11.088	4,466,008	0.09	73, 147,117	523,387
5	β- Arabinopyranose	11.288	3,276,958	0.06	73, 117,147, 217	91,696,778
6	d-(+)-Xylose	12.047	5,553,990	0.11	204, 73, 217, 191	135,191
7	Shikimic acid	13.114	1,769,681,263	37.73	204, 147, 73	8742
8	β-D-Galactofuranose	13.439	88,850,203	1.89	217, 73, 147, 191	250,139,516
9	Gallic acid	14.606	243,045,871	5.18	73, 281, 458	519,814
10	*Myo*-inositol	15.906	177,915,111	3.79	73, 217, 305, 147, 191	520,232
11	Sarotrhin	26.150	119,692,822	2.55	73, 575, 590, 487	5,386,960

RT- retention time.

cancer cells and the ability of cells to migrate. However, the molecular mechanisms involved in these processes have not yet been elucidated, and additional specific studies are required. Phenolic compounds present in the fraction, such as GA and sarothrin, may be responsible for the apoptotic action and decrease in viability and migration of B16F10 tumor cells. In addition, it is necessary to evaluate the pequi fraction on normal skin cells to assess its selectivity on cell phenotypes, as well as establish cytotoxic and antiproliferative effects. Thus, our study offers promising perspectives, providing a basis for deepening the applications of the pequi byproduct as a source of biological molecules used in the fight against cancer.

Ethics approval

All applicable institutional and/or national guidelines for the care and use of animals were followed.

CRediT authorship contribution statement

Jéssica Nayara Basílio Silva: Conceptualization. **Victor Hugo Dantas Guimarães:** Writing – original draft, Formal analysis, Visualization. **Barbhara Mota Marinho:** Writing – original draft. **Amanda Souto Machado:** Formal analysis, Visualization. **Amanda Rodrigues Santos:** Formal analysis, Visualization. **Ludmilla Regina de Souza David:** Conceptualization, Writing – review & editing. **Geraldo Aclécio Melo:** Conceptualization, Writing – review & editing. **Alfredo Maurício Batista de Paula:** Writing – review & editing. **Sérgio Henrique Sousa Santos:** Writing – review & editing.

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.

Funding

The present study was partially supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Brazil), process number CRA-DGE-00,027–13; Coordenadoria de Aperfeiçoamento do Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico (CNPQ).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100273.

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