Revista Colombiana de Cancerología

Octubre - Diciembre / 2023

Rev Colomb Cancerol. 2023;27(4):433-442

https://doi.org/10.35509/01239015.979

ARTÍCULO ORIGINAL

Aqueous extract of Andean berry (*Vaccinium meridionale Swartz*) promotes antiproliferative effect of 5-fluorouracil and leucovorin treatment with or without oxaliplatin and inhibits metastatic potential in colon adenocarcinoma cells

El extracto acuoso de la baya andina (*Vaccinium meridionale Swartz*) promueve el efecto antiproliferativo del tratamiento con 5-fluorouracilo y leucovorina con o sin oxaliplatino, en las células SW480 y SW620 e inhibe el potencial metastásico de células de adenocarcinoma de colon

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Fecha de sometimiento: 02/06/2023
Fecha de aceptación: 19/10/2023
Disponible en internet: 29/12/2023

Citation:

Zapata-Londoño MB, Lozano-Casabianca GA, Landázuri P, Arango-Varela SS, Maldonado-Celis ME. Aqueous extract of Andean berry (*Vaccinium meridionale Swartz*) promotes antiproliferative effect of 5-fluorouracil and leucovorin treatment with or without oxaliplatin and inhibits metastatic potential in colon adenocarcinoma cells. Rev Col Cancerol. 2023;27(4):433-42. https://doi.org/10.35509/01239015.979

Conflicts of interest:

The authors declare no conflict of interest.

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Resumen

Objetivo: Determinar el efecto de un extracto acuoso de la baya andina (*Vaccinium meridionale Swartz*) solo o en combinación con 5-fluorouracilo (5-FU), leucovorina y oxaliplatino sobre proliferación, adhesión y potencial metastásico de líneas celulares de adenocarcinoma de colon.

Métodos: Se utilizaron el ensayo de sulforodamina B, para determinar la viabilidad celular, y la tinción con cristal violeta, para evaluar la adhesión y la eficiencia de clonación de las células SW480 y SW620. Se emplearon estuches comerciales para evaluar la habilidad de migración, invasión y los niveles de metaloproteinasas 2, 7 y 9 en la línea celular SW620.

Resultados: El extracto de la baya andina (30 % v/v), combinado con los medicamentos evaluados, mostró un mayor efecto sobre la viabilidad de las células SW480 y SW620, en comparación con los medicamentos solos. La eficiencia de clonación de ambas líneas celulares fue similar después del tratamiento con los medicamentos o con la combinación de estos con el extracto. El tratamiento con el extracto disminuyó la adhesión de la línea celular SW480, así como también la migración, y la invasión de las células SW620. Adicionalmente, los medicamentos combinados con el extracto acuoso mostraron un mayor efecto inhibitorio sobre la migración e invasión de las células SW620, en comparación con los medicamentos solos. El extracto disminuyó los niveles de MMP-9 de las células SW620.

Conclusiones: El extracto acuoso de baya andina, o agraz, promovió el efecto del 5-FU, oxaliplatino y leucovorina sobre la proliferación de las células SW480 y SW620, además mostró un potencial antimetastásico sobre las células SW620.

Palabras clave: *Vaccinium*, neoplasias del colon, metástasis de la neoplasia, fluorouracilo, leucovorina, oxaliplatino, frutas, línea celular tumoral, fenoles.

Abstract

Objective: To determine the effect of the aqueous extract of Andean berry (*Vaccinium meridionale Swartz*) alone or in combination with 5-fluorouracil (5-FU), leucovorin (LEU), and oxaliplatin (OXA) on proliferation, adhesion, and metastatic potential in colon adenocarcinoma cell lines.

Methods: Sulforhodamine B assay was used to evaluate cell viability, and crystal violet staining was employed to assess the adhesion and cloning efficiency of SW480 and SW620 cells. Commercial kits were used to evaluate the migration, invasion capacity, and levels of matrix metalloproteinases-2, -7, and -9 in the SW620 cell line.

Results: The Andean berry extract (30% v/v) combined with the tested drugs showed a greater inhibitory effect on the viability of SW480 and SW620 cells than the drugs alone. The cloning efficiency of both cell lines was similar after treatment with drugs alone or combined with the extract. The extract decreased the adhesion of SW480 cells and also the migration and invasion of SW620 cells. Moreover, the drugs combined with the aqueous extract showed a greater inhibitory effect on the migration and invasion of SW620 cells than the drugs alone. The extract decreased the matrix metalloproteinase-9 (MMP-9) level of SW620 cells.

Conclusions: The aqueous extract of Andean berry promoted the antiproliferative effect of the combination of 5-FU, LEU, and OXA in SW480 and SW620 cells while showing a potential antimetastatic effect in SW620 cells.

Keywords: *Vaccinium*, colonic neoplasms, neoplasm metastasis, fluorouracil, leucovorin, oxaliplatin, fruit; cell line, tumor; phenols.

Introduction

Colorectal cancer (CRC) is the third most common cancer and has one of the highest mortality rates in the world (1). Different epidemiological studies have demonstrated the role of dietary habits and lifestyle in the development of sporadic CRC, which corresponds to 80 to 85% of cases (2,3). Risk factors include alcohol consumption, red and processed meat intake, obesity, and chronic inflammatory diseases of the colon (2). In contrast, consumption of whole grains, fiber, fruits, and vegetables has been associated with a lower risk of developing sporadic CRC (2).

Because this type of cancer is not detected early in many cases, at the time of diagnosis, 35% of patients present metastasis (4,5); in these cases, the survival rate is lower compared to localized CRC cases (6). The process of metastasis requires cell adhesion to the extracellular matrix, migration, angiogenesis, and invasion of tumor cells (7). Metastasis involves enzymes such as matrix metalloproteinases (MMPs), which promote invasion and angiogenesis by degrading components of the extracellular matrix and basement membrane; MMPs associated with these processes include matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-7 (MMP-7), matrix metalloproteinase-9 (MMP-9), and matrix metalloproteinase-12 (MMP-12) (8).

For the treatment of CRC in more advanced stages, there exist several therapeutic strategies, such as chemotherapy, targeted therapy, or immunotherapy (9). The three most used treatment schemes during chemotherapy are 5-fluorouracil (5-FU) combined with leucovorin (LEU) and oxaliplatin (OXA) (FOLFOX); 5-FU, LEU, and irinotecan (FOLFIRI); and capecitabine combined with OXA (9).

Although the above treatments may increase the survival rate of patients, they have shown low response rates and caused side effects and toxicity in different organs, such as the heart and liver (6,10,11). 5-FU can cause mucositis, neutropenia, and leukopenia (12); OXA causes acute neurotoxicity and chronic cumulative sensory neuropathy (13), whereas irinotecan is associated with myelosuppression, diarrhea, nausea, vomiting, and alopecia (14).

Therefore, it has been proposed that chemoprevention using natural agents could be used not only to prevent the development of CRC but also to be administered in combination with antineoplastic agents to decrease their effective dose, reduce side effects, and improve efficacy (15).

Berries belonging to the *Vaccinium* family, including cranberry, lingonberry, and Andean berry, are rich in phenolic compounds and have shown chemopreventive potential against CRC. Juice extracts and organic-soluble extract made from cranberry were found to decrease cell viability in the Caco-2, HT-29, HCT-16, and LS-513 colon cancer cell lines (16-18).

Borowiec *et al.* (19) analyzed bilberry juice extract and found that it inhibited cell viability in Caco-2 cells at 400 µg dry mass/ml after 48 h of treatment. Phenolic fractions from lingonberry showed antiproliferative activity against HT-29 cells at a concentration of 0.05 mg/ml (20).

The most abundant phenolics in the Andean berry (*Vaccinium meridionale Swartz*) are anthocyanins, phenolic acids, flavonoids, and tannins (21). The anticancer activity of Andean berry has been demonstrated in several studies. In one of them, the nectar prepared with this berry showed antiproliferative activity against colon adenocarcinoma cells (SW480) (22). Likewise, the aqueous extract of this berry inhibited the growth and cloning efficiency of SW480 colon adenocarcinoma cells and their metastatic derivative SW620 cell line (23).

This study aimed to evaluate the antiproliferative and antimetastatic effects of the aqueous extract of Andean berry alone or in combination with 5-FU, LEU, and OXA.

Methodology

Drugs

Oxaliplatin (OXA) (Cat #O9512) and 5-FU (Cat #F6627) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethylsulfoxide (DMSO). Folinic acid calcium salt (Cat #sc-252837) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA) and dissolved in ultra-pure water.

Reagents

Dulbecco's Modified Eagle Medium (DMEM), acetic acid, trichloroacetic acid, Tris solution, methanol, ethanol, phosphate-buffered saline, fetal bovine serum, glucose, L-glutamine, penicillin, streptomycin, and non-essential amino acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). ITS media supplement (10 µg/ml insulin, 5 µg/ml transferrin, and 5 ng/ml selenium) and sulforhodamine B were obtained from Invitrogen (Carlsbad, CA, USA), and crystal violet was purchased from Albor Químicos (Bogotá, Colombia).

Preparation of aqueous extract

The extract was prepared with fresh and ripe berries (black-purple color) from the municipality of Retiro (Antioquia, Colombia) at 2,175 m above sea level (6°08'06''N; 75°25'03''W) and with an average temperature of 16 °C. The material was registered with the voucher number ILS14050070. The berries were washed, disinfected with sodium hypochlorite (100 ppm), washed again with plenty of water, and then subjected to commercial blender for 2 min at 2,500 rpm. The pulp obtained was lyophilized in a vacuum chamber under pressure 4.27 + 0.5 mm Hg at a temperature of -50°C and finally stored at -20°C. The extract was prepared by mixing 6 g of lyophilizate of the Andean berry and 100 ml of water, subsequently sonicated for 3 h, strained, and filtered using a 0.22 µm filter (23).

Cell culture

The SW480 (colon adenocarcinoma) and SW620 (colon adenocarcinoma from metastatic tissue) cell lines were obtained from the European Collection of Animal Cell Culture (ECACC, Salisbury, UK). These cells were maintained at 37°C with 5% CO_2 in DMEM supplemented with glucose (25 mm), L-glutamine (2 mm), penicillin (100 U/ml), streptomycin (100 µg/ml), ITS media supplement, fetal calf serum (FCS) (3%) previously inactivated at 56°C, and non-essential amino acids (1%).

Cell viability analysis

The effect of the extract in combination with 5-FU, LEU, and OXA on cell viability was determined using the sulforhodamine B colorimetric method (23). The SW480 and SW620 cells were seeded in a 96-well plate at a density of 150,000 and 180,000 cells/ml; after 24 h of incubation at 37 ºC and 5% CO_2, treatments were added. After 48 h of treatment, the sulforhodamine B assay was carried out, for which the cells were fixed with trichloroacetic acid (50% v/v) for 1 h at 4°C, then washed with water; the plates were allowed to air dry. Subsequently, the cells were stained with sulforhodamine B (0.4%) for 30 min, washed with acetic acid (1% v/v), and left to dry overnight. Finally, they were incubated with 10 mm of Tris solution (pH 10.5) at 55 rpm. Optical density (OD) was measured at 490 nm in a microplate reader (Bio-Rad iMARK, Berkeley, CA,

USA). The half-maximal inhibitory concentration (IC_{50}) of the drugs was determined in SW480 and SW620 cells. 5-FU was evaluated at concentrations corresponding to 400, 200, 100, and 50 µm; LEU was assessed at a concentration of 80 µm, and OXA at concentrations corresponding to 200, 100, 50, and 25 µm. The IC_{50} of the extract evaluated in SW480 and SW620 cells was determined in a previous study (23). After establishing the IC_{50} value of each drug in both cell lines, the effect of the Andean berry extract on cell viability was evaluated at a concentration of 30% v/v combined with 5-FU+LEU and 5-FU+LEU+OXA at three different concentrations corresponding to CI_{50}, ½ $CI_{50,}$ and ¼ CI_{50}. The final volume of the extract was 60 µl per well. After 48 h of treatment, optical density was measured at 490 nm in a microplate reader (Bio-Rad iMARK, Berkeley, CA, USA) to determine the percentage of cell-growth inhibition using the following formula: [1-(ODt/ODc) * 100], where ODt is the optical density of treated cells, and ODc is the optical density of control cells. Untreated cells with culture media were assayed as control.

Cloning efficiency

The SW480 and SW620 cells were seeded in 12-well dishes at a density of 150 cells/well and 180 cells/well in 3% DMEM, respectively, and incubated for 24 h at 37°C with 5% CO_2. Subsequently, Andean berry extract (30% v/v), 5-FU, and LEU were added, with or without OXA. After 48 h of treatment, the medium was removed; the cells were washed with poly (butylene succinate) (PBS) and incubated with a new medium for 7 days. The medium was changed every 48 h. After this incubation, the medium was removed, and the cells were washed with PBS and fixed with Carnoy's solution (methanol-acetic acid 3:1) for 1 h. Subsequently, the cells were stained with crystal violet (0.5%) for 10 min and then washed with water and allowed to dry. Finally, the number of colonies (50 or more cells) formed throughout the well was counted under an inverted microscope (Olympus, Tokyo, Japan). The percentage of relative cloning efficiency (RCE), an indicator of antiproliferative effect, was determined as follows: RCE = (number of colonies/number of control colonies) x 100 (24). The control consisted of untreated cells. The evaluated concentrations of 5-FU and OXA were 95.5 mm and 15.5 mm, respectively, for SW480 cells. For SW620 cells, the concentrations of 5-FU and OXA were 78.5 mm and 5.5 mm, respectively. The above concentrations were also used for the assays detailed below.

Adhesion assay

The SW480 and SW620 cells at a density of 700,000 cells/ml were pretreated with the aqueous extract of Andean berry and/or drugs, and after 1 h of incubation, they were seeded in 12-well plates coated with type I collagen and incubated at 37°C and 5% CO_2. After 24 h of treatment, non-adhered cells were washed with PBS, and adhered cells were fixed for 1 h with Carnoy's solution and subsequently stained with crystal violet (0.02%); after 10 min, ethanol (70%) was added at room temperature (RT). Finally, OD was measured at 570 nm in a microplate reader (Bio-Rad iMARK, Berkeley, CA, USA). Adhesion ability was determined as follows: Adhesion ability = ODt/ODc x 100 (7). The control consisted of untreated cells.

Determination of migration capacity

The migration capacity of SW620 cells was determined using a commercial kit (Trevigen RAB3465-096K, Minneapolis, MN, USA), following the manufacturer's instructions. Cells were maintained in a serum-free medium 24 h before the assay and subsequently seeded in the upper chamber at a density of 50,000 cells/well in a serum-free medium; in the lower chamber, DMEM supplemented with 10% FCS was added. Subsequently, the drugs were added with or without the aqueous extract of Andean berry dissolved in the medium and incubated at 37°C with 5% CO_2. After 48 h of treatment, the cells were stained with Calcein-M. Subsequently, fluorescence reading was performed at 485 nm excitation and 528 nm emission in a multimode microplate reader (Biotek Synergy HTX, Agilent, Santa Clara, CA, USA). The control consisted of untreated cells. Data are presented as relative fluorescence units (RFU), directly proportional to the migration capacity.

Determination of invasion ability

The invasiveness of SW620 cells was determined using a commercial kit (Trevigen RAB3455-096K, Minneapolis, MN, USA), following the manufacturer's instructions. Cells were maintained in a serum-free medium 24 h before the assay; subsequently, basement membrane extract was added to the chamber wells and incubated at 37°C with 5% CO_2. After 24 h, cells were seeded in the upper chamber at a density of 50,000 cells/well in a serum-free medium, and DMEM supplemented with 10% FCS was added to the lower chamber. Subsequently, the

drugs were added with or without the Andean berry extract dissolved in the medium and incubated at 37°C with 5% CO_2. After 48 h of treatment, cells were stained with Calcein-M, after which fluorescence reading was performed at 485 nm excitation and 528 nm emission in a multimode microplate reader (Biotek Synergy HTX, Agilent, Santa Clara, CA, USA). The control consisted of untreated cells. Data are presented as RFU, directly proportional to the invasion capacity.

Determination of MMP-2, MMP-7, and MMP-9 levels

MMP-2, MMP-7, and MMP-9 levels were assessed by sandwich ELISA commercial kits (RAB0365, RAB0369, and RAB0372, respectively) (Sigma-Aldrich, St. Louis, MO, USA). SW620 cells were seeded at a density of 180,000 cells/well in 96-well plates; after 24 h of incubation, the Andean berry aqueous extract was added alone or in combination with 5-FU+LEU and 5-FU+LEU+OXA. After 48 h of treatment, supernatants were collected and centrifuged at 5,000 g for 10 min at RT. MMP-2, MMP-7, and MMP-9 levels were determined according to the manufacturer's instructions. OD was measured at 450 nm in a microplate reader (Bio-Rad iMARK, Berkeley, CA, USA).

Statistical analysis

Data are presented as mean ± standard error of the mean or median and interquartile range from three independent experiments. All assays were carried out in triplicate, and differences between treatments were analyzed by one-way analysis of variance (ANOVA), followed by unpaired Tukey test or Kruskal-Wallis test followed by Dunn's test. The statistical analysis was performed using GraphPad Prism 9.1. Differences were considered significant if p-value was <0.05.

Results

Effect of Andean berry and drugs on cell viability

Aqueous extract of Andean berry has been shown to decrease the cell viability of SW480 and SW620 cells at a concentration corresponding to 30% v/v (18). The percentage of cell-growth inhibition of SW620 and SW480 cells was higher after treatment with 5-FU+LEU+OXA (table 1). The viability of SW480 and SW620 cells was significantly lower after treatment with the combination of these drugs and the extract than with treatments without the extract. The effect on viability between different treatments without the extract did not show significant differences (table 1).

Table 1. Effect of 5-FU+LEU and 5-FU+LEU+OXA, alone or in combination with Andean berry extract, on cell viability

	% inhibition of cell viability	
	SW480	SW620
5-FU+LEU	23.9%[a]	38.9%[a]
5-FU+LEU+A	77.0%[b]	85.5%[b]
5-FU+LEU+OXA	55.4%[a]	51.9%[a]
5-FU+LEU+OXA+A	82.5%[b]	91.9%[b]

[a,b] Different letters in the same column indicate significant differences ($p<0.05$). The cells were exposed to 5-FU+LEU and 5-FU+LEU+OXA, with or without Andean berry extract (30% v/v), for 48 h. 5-FU and OXA were evaluated at concentrations corresponding to 95.5 mm and 15.5 mm, respectively, for SW480 cells; for SW620 cells, these concentrations were 78.5 mm and 5.5 mm, respectively. LEU was evaluated at a concentration of 80 mm for both cell lines. The control consisted of untreated cells. Data are expressed as mean ± standard error of the mean from three independent experiments. 5-FU: 5-fluorouracil; LEU: leucovorin; A: Andean berry extract; OXA: oxaliplatin.

The CI_{50} value of 5-FU+LEU and 5-FU+LEU+OXA for SW480 cells was 381.7 mm and 62.8 mm, respectively, and 313.9 mm and 22.09 mm for SW620 cells, respectively.

Significant differences were observed after treatment with the drugs at different concentrations, with or without the extract, compared to the control in both cell lines (data not shown). However, there were no significant differences between drug treatments at different concentrations in combination with the Andean berry extract (30% v/v); therefore, the lower concentrations of 5-FU+LEU and 5-FU+LEU+OXA were used for the assays described below.

Effect of Andean berry extract and drugs on cloning efficiency

A previous study demonstrated the effect of Andean berry extract on cloning efficiency; therefore, the effect of the extract combined with the drugs was evaluated (18). Andean berry extract combined with 5-FU+LEU or 5-FU+LEU+OXA inhibited colony formation in SW480 and SW620 cells. The percentage of RCE after the different treatments showed significant differences compared to the control; on the contrary, cloning efficiency did not show differences after treatment with the drugs alone compared to the drugs combined with the extract in both cell lines (table 2).

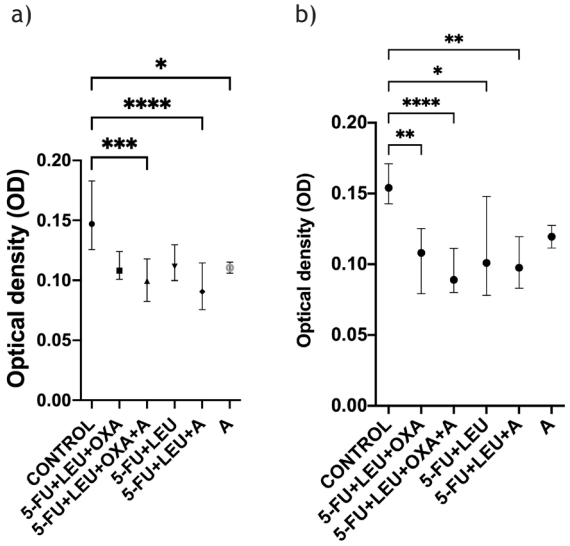
Table 2. Effect of 5-FU+LEU and 5-FU+LEU+OXA, alone or in combination with Andean berry extract, on cloning efficiency

	SW480	SW620
Treatment	RCE	RCE
5-FU+LEU	0[a]	0[a]
5-FU+LEU+A	0[a]	0[a]
5-FU+LEU+OXA	0[a]	0[a]
5-FU+LEU+OXA+A	0[a]	0[a]
Control	100%[b]	100%[b]

[a,b] Different letters in the same column indicate significant differences ($p<0.05$). The cells were exposed to 5-FU+LEU and 5-FU+LEU+OXA (at the same concentrations described in table 1), with or without Andean berry extract (30% v/v), for 48 h. The control consisted of untreated cells. Data are expressed as mean ± standard error of the mean from three independent experiments. 5-FU: 5-fluorouracil; LEU: leucovorin; A: Andean berry extract; OXA: oxaliplatin; RCE: Relative cloning efficiency.

Effect of Andean berry extract and drugs on cell adhesion

The adhesion of SW480 and SW620 was lower after the treatment with Andean berry extract (30% v/v) in combination with 5-FU+LEU, with or without OXA, compared to the control sample (figures 1a, 1b). The treatment with 5-FU+LEU did not induce a decrease in the cell adhesion of SW480 cells; on the contrary, the combination of these drugs with the extract lowered SW480 adhesion. However, there were no differences in SW480 cell adhesion between the treatment with 5-FU+LEU+extract and extract alone. After the treatment with extract alone, SW480 adhesion was lower (figure 1a); on the contrary, there were no significant differences in SW620 adhesion compared



5-FU: 5-fluorouracil; LEU: leucovorin; A: Andean berry extract; OXA: oxaliplatin.

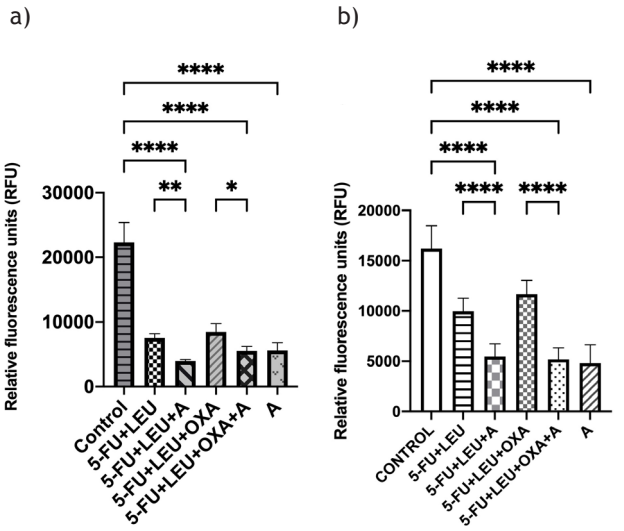
Figure 1. Effect of Andean berry extract in combination with 5-FU+LEU and 5-FU+LEU+OXA on adhesion in SW480 **(a)** and SW620 **(b)** cell lines. The cells were exposed to 5-FU+LEU and 5-FU+LEU+OXA, with or without Andean berry extract (30% v/v), for 48 h. 5-FU and OXA were evaluated at concentrations corresponding to 95.5 mm and 15.5 mm, respectively, for SW480 cells; for SW620 cells, these concentrations were 78.5 mm and 5.5 mm, respectively. LEU was evaluated at a concentration of 80 mm for both cell lines. Data are expressed as median ± interquartile range from three independent experiments. *$p<0.05$, **$p<0.005$, ***$p<0.0009$, ****$p<0.0001$ (Kruskal-Wallis test and Dunn's test).

Effect of the Andean berry extract and drugs on cell migration

The effect of Andean berry extract combined with 5-FU+LEU, with or without OXA, on cell migration in SW620 cells was evaluated. Both treatments, 5-FU+LEU and 5-FU+LEU+OXA, decreased cell migration compared to the control, which was statistically significant ($p<0.0001$). Similarly, cell migration after treatment with the extract combined with 5-FU+LEU or 5-FU+LEU+OXA was significantly lower compared to the control sample ($p<0.0001$). Cell invasion reduction was greater after combining the extract with the drugs than in the case of drugs alone. Moreover, the extract alone reduced SW620 migration compared to the control sample ($p<0.0001$), and this reduction was higher compared to combined 5-FU, LEU, and OXA ($p<0.05$) (figure 2a).

Effect of the Andean berry extract and drugs on cell invasion

The effect of Andean berry extract combined with 5-FU+LEU and 5-FU+LEU+OXA on cell invasion in SW620 cells was evaluated. 5-FU+LEU, with or without OXA, induced a significant decrease in cell invasion compared to the control sample after 48 h of treatment ($p<0.0001$ and $p<0.0001$, respectively). Also, the extract in combination with 5-FU+LEU or 5-FU+LEU+OXA significantly decreased cell invasion compared to the control sample ($p<0.0001$). The decrease in cell invasion was greater after combining the extract with the drugs compared to the drugs alone. Cell invasion after treatment with the extract alone showed significant differences compared to the control sample ($p<0.0001$) and the combined 5-FU+LEU with or without OXA ($p<0.0001$) (figure 2b).



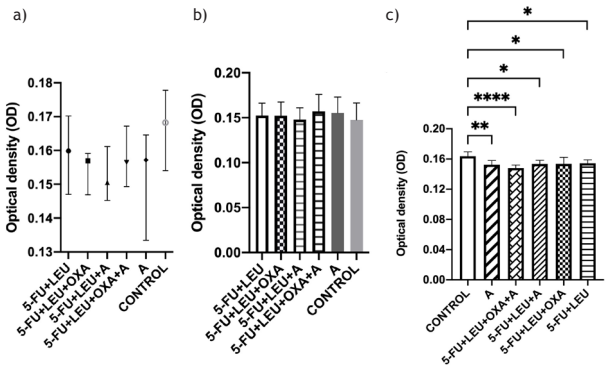
5-FU: 5-fluorouracil; LEU: leucovorin; A: Andean berry extract; OXA: oxaliplatin.

Figure 2. Effect of Andean berry extract in combination with 5-FU+LEU and 5-FU+LEU+OXA on migration **(a)** and invasion **(b)** in SW620 cells. The cells were exposed to 5-FU+LEU and 5-FU+LEU+OXA, with or without Andean berry extract (30% v/v), for 48 h. 5-FU, LEU, and OXA were evaluated at concentrations corresponding to 78.5 mm, 5.5 mm, and 80 mm, respectively. Data are expressed as mean ± standard error of the mean from three independent experiments. *$p<0.05$, **$p<0.005$, ****$p<0.0001$.

Effect of the Andean berry extract and drugs on MMP-2, MMP-7, and MMP-9 levels

The effect of Andean berry extract combined with 5-FU+LEU and 5-FU+LEU+OXA on the levels of MMP-2, MMP-7, and MMP-9 in SW620 supernatants was evaluated. MMP-2 and MMP-7 levels after treatment with 5-FU+LEU, with or without OXA, did not show significant differences compared to the control sample. Similarly, the combination of the extract with 5-FU+LEU or 5-FU+LEU+OXA did not induce changes in the levels of MMP-2 (figure 3a) and MMP-7 (figure 3b) compared to the control sample. The extract alone did not have an effect on MMP-2 and MMP-7 levels in SW620 supernatants compared to the control (figures 3a, 3b).

On the contrary, the MMP-9 level was lower after the treatment with 5-FU+LEU and FU+LEU+OXA, with or without the extract, compared to the control sample. Also, the extract alone induced a reduction in MMP-9 levels in SW620 cells. MMP-9 levels were similar between the different treatments compared to the control sample (figure 3c).



5-FU: 5-fluorouracil; LEU: leucovorin; A: Andean berry extract; OXA: oxaliplatin.

Figure 3. Effect of Andean berry extract in combination with 5-FU+LEU and 5-FU+LEU+OXA on the levels of MMP-2 **(a)**, MMP-7 **(b)**, and MMP-9 **(c)** of SW620 cells. The cells were exposed to 5-FU+LEU and 5-FU+LEU+OXA, with or without Andean berry extract (30% v/v), for 48 h. 5-FU, LEU, and OXA were evaluated at concentrations corresponding to 78.5 mm, 5.5 mm, and 80 mm, respectively. Data are expressed as mean ± standard error of the mean from three independent experiments. For the MMP-2 analysis, Kruskal-Wallis and Dunn's tests were carried out, and data are represented as median ± interquartile range. *$p<0.05$, **$p<0.005$, ****$p<0.0001$.

Discussion

Phytochemicals from plants and fruits have been shown to maximize the effect of and reduce resistance to antitumor drugs; therefore, combining antineoplastic drugs with fruits or their components has been investigated as a new potential strategy for colorectal cancer treatment (25). Andean berry is a fruit that has shown antiproliferative potential; however, there are no reports about the effect of the combination of this berry with drugs used in colorectal cancer therapy.

In this study, Andean berry extract promoted the antiproliferative effect of 5-FU+LEU and 5-FU+LEU+OXA in SW480 and SW620 cells. This antiproliferative effect has been shown in another berries, including blueberry (*V. corymbosum*). Blueberry inhibited the growth of colon cancer cell lines HT-29 and HCT116 at concentrations corresponding to 89.96 ± 0.05 and 90 ± 0.05 µg/ml, respectively, after 48 h of treatment (26).

Tolba *et al*. (27) found that pterostilbine, a component from berries, combined with 5-FU, induced a greater cytotoxic effect than 5-FU alone against HCT116 and Caco-2 colon cancer cell lines. 5-FU showed an IC_{50} value of 46.8 ± 2.5 µm and 4.3 ± 0.85 µm in Caco-2 and HCT116 cells, respectively; these values were higher compared to the values shown by pterostilbine/5-FU corresponding to IC_{50} of 2.44 ± 0.16 µm and 1.07 ± 0.01 µm in Caco-2 and HCT116 cells, respectively.

Black raspberry anthocyanins (50 µg/ml) increased the ability of 5-FU (16 µm) to reduce the proliferation of human colorectal cancer cell lines corresponding to SW480 and Caco-2 (28).

In this study, combined 5-FU and LEU, with or without OXA and Andean berry extract, induced a reduction in SW620 adhesion; however, there were no differences between the combination of drugs alone compared to the drugs combined with the extract, which indicates that the effect observed on SW620 adhesion could be attributed to the drugs. Similarly, the extract did not promote the effect of the tested drugs on SW480 adhesion.

The potential antimetastatic effect of the extract was evaluated in SW620 cells, considering that this cell line was derived from a metastatic lymph node. The Andean berry extract alone or in combination with the studied drugs decreased migration and invasion in SW620 cells, indicating its potential to inhibit processes that promote metastasis. Similar results were shown by Behrendt *et al*. (29), who carried out a randomized, placebo-controlled, crossover study with 34 participants who were given an anthocyanin-rich juice made from red grape (80%) and bilberry or placebo juice. Participants took 0.33 l of juice or placebo daily for 28 days, and blood samples were taken before and after intervention periods. Subsequently, anthocyanins and their metabolites were extracted from plasma and solved in culture media to treat Caco-2 and HT-29 cell lines (human colon cancer cells) with or without 5-FU for 36 h. Plasma-isolated anthocyanins and their metabolites decreased migration in the HT-29 cell line and enhanced the anti-migration effect of 5-FU.

Brown *et al*. (30) showed that digested and fermented lingonberry extract inhibited the invasion capacity of HT115 colon cancer cell line at concentrations corresponding to 3 µg/ml and 16 µg/ml gallic acid equivalent (GAE).

It is possible that the Andean berry extract inhibits targets different from those of OXA and 5-FU and that these differences could enhance the antiproliferative and antimetastatic effect of these drugs. Berries have been shown to inhibit proteins involved in cell adhesion, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), as well as proteins related to invasion (31,32).

Considering that MMPs are involved in the invasion process, the effect of the Andean berry extract alone or in combination with drugs on MMP-2, MMP-7, and MMP-9 levels was evaluated. Only the MMP-9 level was lower after treatment with the extract alone or combined with the drugs.

In conclusion, aqueous extract of Andean berry shows a negative impact on the metastatic potential of SW620 cells and enhances the antiproliferative effect of 5-FU, LEU, and OXA in SW480 and SW620 cells. As such, including the Andean berry in the diet could offer potential benefits for colorectal cancer patients. Nevertheless, further studies using *in vivo* models are necessary to fully elucidate its anticancer potential.

ACKNOWLEDGMENTS

This research was funded by the *Ministerio de Ciencia, Tecnología e Innovación* (MinCiencias), in alliance with the *Ministerio de Educación Nacional* and *Ministerio de Comercio, Industria y Turismo*, through the Scientific Ecosystem component of the *Colombia Científica* program (*Alianza NanoBioCáncer* Code FP44842-211-2018, Project No. 58580), the post-doctoral internship program of MinCiencias (Grant number: 143-2020), and the of Sustainability Strategy for Research Groups 2020-2021 of the Committee for the Development of Research (CODI, for its acronym in Spanish) at the *Universidad de Antioquia*, Medellín, Colombia.

References

1. International Agency for Research on Cancer. Population fact sheets. Cancer Today [Internet] [cited 14 Oct 2022]. Available from: https://gco.iarc.fr/today/fact-sheets-populations

2. World Cancer Research Fund/American Institute for Cancer Research. Diet, nutrition, physical activity and colorectal cancer. Revised 2018 [Internet]. Available from: https://www.wcrf.org/wp-content/uploads/2021/02/Colorectal-cancer-report.pdf

3. Fischer J, Walker LC, Robinson BA, Frizelle FA, Church JM, Eglinton TW. Clinical implications of the genetics of sporadic colorectal cancer. ANZ J Surg. 2019;89(10):1224-9. https://doi.org/10.1111/ans.15074

4. Lee RM, Cardona K, Russell MC. Historical perspective: Two decades of progress in treating metastatic colorectal cancer. J Surg Oncol. 2019;119(5):549-63. https://doi.org/10.1002/jso.25431

5. Piawah S, Venook AP. Targeted therapy for colorectal cancer metastases: A review of current methods of molecularly targeted therapy and the use of tumor biomarkers in the treatment of metastatic colorectal cancer. Cancer. 2019;125(23):4139-47. https://doi.org/10.1002/cncr.32163

6. Advani S, Kopetz S. Ongoing and future directions in the management of metastatic colorectal cancer: update on clinical trials. J Surg Oncol. 2019;119(5):642-52. https://doi.org/10.1002/jso.25441

7. Juan TK, Liu KC, Kuo CL, Yang MD, Chu YL, Yang JL, *et al*. Tetrandrine suppresses adhesion, migration and invasion of human colon cancer SW620 cells via inhibition of nuclear factor-κB, matrix metalloproteinase-2 and matrix metalloproteinase-9 signaling pathways. Oncol Lett. 2018;15(5):7716-24. https://doi.org/10.3892/ol.2018.8286

8. Mustafa S, Koran S, AlOmair L. Insights into the role of matrix metalloproteinases in cancer and its various therapeutic aspects: a review. Front Mol Biosci. 2022;9:896099. https://doi.org/10.3389/fmolb.2022.896099

9. Alfaro Alfaro ÁE, Murillo Castillo B, Cordero García E, Tascón J, Morales AI. Colon cancer pharmacogenetics: a narrative review. Pharmacy (Basel). 2022;10(4):95. https://doi.org/10.3390/pharmacy10040095

10. Sara JD, Kaur J, Khodadadi R, Rehman M, Lobo R, Chakrabarti S, *et al*. 5-fluorouracil and cardiotoxicity: a review. Ther Adv Med Oncol. 2018;10:1-13. https://doi.org/10.1177/1758835918780140

11. El Chediak A, Haydar AA, Hakim A, Massih SA, Hilal L, Mukherji D, *et al*. Increase in spleen volume as a predictor of oxaliplatin toxicity. Ther Clin Risk Manag. 2018;14:653-7. https://doi.org/10.2147/TCRM.S150968

12. Arshad U, Ploylearmsaeng SA, Karlsson MO, Doroshyenko O, Langer D, Schömig E, *et al*. Prediction of exposure-driven myelotoxicity of continuous infusion 5-fluorouracil by a semi-physiological pharmacokinetic-pharmacodynamic model in gastrointestinal cancer patients. Cancer Chemother Pharmacol. 2020;85(4):711-22. https://doi.org/10.1007/s00280-019-04028-5

13. Kang L, Tian Y, Xu S, Chen H. Oxaliplatin-induced peripheral neuropathy: clinical features, mechanisms, prevention and treatment. J Neurol. 2021;268(9):3269-82. https://doi.org/10.1007/s00415-020-09942-w

14. Bailly C. Irinotecan: 25 years of cancer treatment. Pharmacol Res. 2019;148:104398. https://doi.org/10.1016/j.phrs.2019.104398

15. Lepore Signorile M, Grossi V, Fasano C, Simone C. Colorectal cancer chemoprevention: a dream coming true? Int J Mol Sci. 2023;24(8):7597. https://doi.org/10.3390/ijms24087597

16. Boivin D, Blanchette M, Barrette S, Moghrabi A, Béliveau R. Inhibition of cancer cell proliferation and suppression of TNF-induced activation of NFKappaB by edible berry juice. Anticancer Res. 2007;27(2): 937-48. PMID: 17465224

17. Vu KD, Carlettini H, Bouvet J, Cote J, Doyon G, Sylvain JF, *et al*. Effect of different cranberry extracts and juices during cranberry juice processing on the antiproliferative activity against two colon cancer cell lines. Food Chem. 2012;132(2):959-67. https://doi.org/10.1016/j.foodchem.2011.11.078

18. Seeram NP, Adams LS, Hardy ML, Heber D. Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. J Agric Food Chem. 2004;52(9): 2512-7. https://doi.org/10.1021/jf0352778

19. Borowiec K, Szwajgier D, Olejnik A, Kowalska K, Targonski Z. Effects of a bilberry preparation on selected cell lines of the digestive system. Czech J Food Sci. 2016;34(4):300-5. https://doi.org/10.17221/375/2015-CJFS

20. Vilkickyte G, Raudone L, Petrikaite V. Phenolic fractions from *Vaccinium vitis-idaea* L. and their antioxidant and anticancer activities assessment. Antioxidants (Basel). 2020;9(12):1261. https://doi.org/10.3390/antiox9121261

21. Maldonado-Celis ME, Arango-Varela SS, Rojano BA. Free radical scavenging capacity and cytotoxic and antiproliferative effects of *Vaccinium meridionale* Sw. against colon cancer cell lines. Rev Cubana Plant Med. 2014;19(2):172-84. Available from: http://scielo.sld.cu/pdf/pla/v19n2/pla06214.pdf

22. Franco Tobón YN, Rojano BA, Alzate Arbeláez AF, Morales Saavedra DM, Maldonado Celis ME. Efecto del tiempo de almacenamiento sobre las características fisicoquímicas, antioxidantes y antiproliferativas de néctar de agraz (*Vaccinium meridionale* Swartz). Arch Lat Nutr. 2016;66(4):261-71. Available from: Available from: https://www.alanrevista.org/ediciones/2016/4/art-1/

23. Arango-Varela SS, Torres-Camargo D, Reyes-Dieck C, Zapata-Londoño MB, Maldonado-Celis ME. Aqueous extract of Andean berry induces apoptosis in human colon cancer cells without mitochondrial damage. J Berry Res. 2021;11(3):377-93. https://doi.org/10.3233/JBR-200684

24. Buch K, Peters T, Nawroth T, Sänger M, Schmidberger H, Langguth P. Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay: a comparative study. Radiat Oncol. 2012;7:1. https://doi.org/10.1186/1748-717X-7-1

25. Rejhová A, Opattová A, Čumová A, Slíva D, Vodička P. Natural compounds and combination therapy in colorectal cancer treatment. Eur J Med Chem. 2018;144:582-94. https://doi.org/10.1016/j.ejmech.2017.12.039

26. Seeram NP, Adams LS, Zhang Y, Lee R, Sand D, Scheuller HS, *et al*. Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. J Agric Food Chem. 2006;54(25):9329-39. https://doi.org/10.1021/jf061750g

27. Tolba MF, Abdel-Rahman SZ. Pterostilbine, an active component of blueberries, sensitizes colon cancer cells to 5-fluorouracil cytotoxicity. Sci Rep. 2015;5:15239. https://doi.org/10.1038/srep15239

28. Li X, Chen L, Gao Y, Zhang Q, Chang AK, Yang Z, *et al*. Black raspberry anthocyanins increased the antiproliferative effects of 5-Fluorouracil and Celecoxib in colorectal cancer cells and mouse model. J Funct Foods. 2021;87(11):104801. https://doi.org/10.1016/j.jff.2021.104801

29. Behrendt I, Röder I, Will F, Mostafa H, Gonzalez-Dominguez R, Meroño T, *et al*. Influence of plasma-isolated anthocyanins and their metabolites on cancer cell migration (ht-29 and caco-2) in vitro: results of the attach study. Antioxidants (Basel). 2022;11(7):1341. https://doi.org/10.3390/antiox11071341

30. Brown E.M, Nitecki S, Pereira-Caro G, McDougall GJ, Stewart D, Rowland I, *et al*. Comparison of in vivo and in vitro digestion on polyphenol composition in lingonberries: Potential impact on colonic health. Biofactors. 2014;40(6):611-23. https://doi.org/10.1002/biof.1173

31. Afrin S, Giampieri F, Gasparrini M, Forbes-Hernandez TY, Varela-López A, Quiles JL, *et al*. Chemopreventive and therapeutic effects of edible berries: a focus on colon cancer prevention and treatment. Molecules. 2016;21(2):169. https://doi.org/10.3390/molecules21020169

32. Kristo AS, Klimis-Zacas D, Sikalidis AK. Protective role of dietary berries in cancer. Antioxidants (Basel). 2016;5(4):37. https://doi.org/10.3390/antiox5040037