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Proceeding Paper

Bioactive Compounds from Andean Berry (*Vaccinium meridionale* Swartz) Juice Inhibited Cell Viability and Proliferation from SW480 and SW620 Human Colon Adenocarcinoma Cells †

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Abstract: The Andean berry is a South American fruit displaying anti-cancer effects. This research aimed to assess the antiproliferative effects of the juice (ABJ) on two human colon cells (SW480/SW620) through viability, proliferation, and Ki67 protein quantification. All treatments (0–30% *v*/*v*) reduced viability (<80%) and proliferation, being 30% most effective (IC_{50}-SW480: 28.19% *v*/*v* ABJ; IC_{50}-SW620: 19.43% *v*/*v* ABJ). All ABJ-treated cells showed a reduced ki67 amount compared to untreated cells. The highest binding affinity (−9.4 kcal/mol) was shown between cyanidin-3-glucoside and caspase 8 in silico. ABJ exerts antiproliferative/inhibitory effects on SW480/SW620 cells, attributable to polyphenol-apoptotic target interactions.

Keywords: Andean berry (*Vaccinium meridionale* Swartz); cell viability; colorectal cancer; phenolic compounds; in silico analysis

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1. Introduction

The Andean berry (*Vaccinium meridionale* Swartz) is a tropical-origin South American fruit commonly cultivated in the Andes, and it is usually consumed as fresh fruit or in typical food preparations such as jams or juices [1]. Despite the reported potential health benefits of this fruit, it is still an underutilized agricultural product with a low marketable perspective and a consumption practically restricted to local areas [2]. Hence, research aiming to valorize the health benefits of this fruit will significantly enhance its consumption and value-added transformation within the food industry.

The major beneficial compounds linked to the health properties of the Andean berry are the phenolic compounds, which potentially target biological mechanisms from chronic non-communicable diseases. For instance, phenolic compounds are known to target key pro-apoptotic proteins such as caspase-3 and caspase-9 and are also involved in the modulation of the canonical inflammation pathway guided by NF-κB. Particularly for the Andean berry, our research group has previously reported that free phenolic compounds such as gallic acid and cyanidin-3-*O*-glucoside targeted inflammatory markers such as CCR5, CCR1, and Toll-like receptor 4 (TLR4) in LPS-stimulated RAW 264.7 macrophages [3]. More recently, it was found that Andean berry juice (ABJ) was successful in reducing cell viability of SW480 cells due to the production of intracellular reactive oxygen species (ROS),

decreased reduced glutathione/oxidized glutathione ratio (GSH/GSSG), and induction of late apoptosis [4]. In an in vivo model of azoxymethane-induced colorectal cancer, ABJ consumption induced Caspase 3/7 activity, reduced aberrant crypt foci number, and decreased local intestinal inflammation [5]. Despite the observed effects, more mechanistic explanations about the impact of ABJ on different colorectal cancer stages are needed. Hence, this research aimed to assess the antiproliferative and cell-viability-inhibition effect of ABJ on two representative human colorectal adenocarcinoma cells (SW480 and SW620). An in silico analysis targeting selected pro-apoptotic markers and representative polyphenols from ABJ was also conducted.

2. Materials and Methods

2.1. Andean Berry Juice (ABJ) Preparation

Fruits were acquired in El Retiro (Antioquia, Colombia), registered in the Herbarium of Universidad de Antioquia (National Registry of Biological Collections #027), washed and disinfected, dried, and ground in a commercial blender. The product was then frozen at −80 °C, and freeze-dried (0.43 mm H_g, −50 °C) using a vacuum chamber (FreeZone Dryer, Labconco, Kansas City, MO, USA). The freeze-dried powders (30 g) were dissolved in a 10% water/sucrose solution to obtain a juice with 11 ºBrix and 4.33 mg citric acid/mL at pH: 3.06. Sonication (42 kHz, 135 W, B3510 sonicator, Ultrasonic Corporation, Boston, MA, USA) was used to ensure a better homogeneity [5].

2.2. Cell Culture

Human colon adenocarcinoma SW480 [SW-480] (ATCC CCL-228) and SW620 [SW-620] (ATCC CCL-227) cancer cells were acquired from American Type Culture Collection (ATCC). These cells were used as representatives of primary (SW480) and secondary (SW620) colorectal tumors. The cells were cultured using high glucose (25 mM) Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic and antimycotic solution (Gibco), and 1% non-essential amino acids. The cells were maintained in a 5% CO_2-humidified atmosphere at 37 °C. During the treatments with ABJ, the cells were cultured in a 3% FBS-supplemented DMEM medium supplemented with 1% Insulin–Transferrin–Selenium (ITS) solution (Gibco).

2.2.1. Cell Viability and Antiproliferative Effect Using Sulforhodamine B Assay

The cell viability was tested using the sulphorhodamine B assay [6]. Briefly, the cells (2×10^4 cells/well; 100 µL) were seeded for 24 h. Then, the cells were treated with ABJ (10, 20, and 30% v/v) for 24 h. Then, the media was replaced with 50 µL of cold trichloroacetic acid, and the plates were incubated for 1 h at 4 °C. The plates were then washed at room temperature (22 ± 1 °C), dried, and 80 µL of sulforhodamine B solution (0.4% w/v) was added. After shaking for 30 min at 80 cycles/min for 30 min, plates were washed 4 times with 1% v/v acetic acid, dried, and 100 µL of tris-HCl were added. After 20 min shaking, the optical density was measured ad 490 nm. Viability was calculated as a percentage of the control cells (untreated SW480 or SW620 cells). The half-inhibitory concentration (IC_{50}) was calculated for each cell line.

The examination of proliferation was conducted as a sulforhodamine B monitoring along time (72 h). The cells (3×10^3 cells/well; 100 µL) were seeded in 96-well plates for 24 h. Then, the media was removed, and the ABJ treatments were added and maintained for 24, 48, and 72 h (1 plate for each time, 2 independent experiments). After each time, sulphorhodamine B staining was conducted to assess cell viability. Results were expressed as an optical density (490 nm) curve against ABJ concentrations over time.

2.2.2. Quantification of Ki67 Protein

The Human Ki67 ELISA kit (ab253221, Abcam, Cambridge, UK) was used to assess the amount of released Ki67 protein. The cells were treated with IC_{50}-ABJ, and the manu-

facturer's instructions were followed. The results were expressed as pg/mL. Untreated cells were used as a control.

2.2.3. In Silico Analysis

Considering a previously reported polyphenolic characterization of ABJ [3–5,7], an in silico analysis was conducted using three representative ABJ anthocyanins and several pro-apoptotic targets. The apoptotic targets (FAS, PDB ID: 3EZQ; Pro-caspase 3, PDB ID: 2J30; Caspase 3: PDB ID: 3KJF; and Caspase 8, PDB ID: 5H31) were downloaded from the Protein Data Bank (https://www.rcsb.org/ (accessed on 21 August 2022)), whereas the anthocyanins' 3D structures (cyanidin-3-galactoside, PubChem CID: 44256700; cyanidin-3-glucoside, PubChem CID: 44256715; delphinidin-3-glucoside, PubChem CID: 443650; and peonidin-3-glucoside, PubChem CID: 443654) were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/ (accessed on 21 August 2022)). The ligands (anthocyanins) were moved to the lower energy consumption mode using the MMFF94 force field using Avogadro v.1.2.015. Then, both proteins and ligands were prepared through solvent elimination and charge addition (Amber ff14SB method), and the residues were protonated using Chimera 1.15 [8]. Molecular docking was performed in AutoDock Vina [9], and the best poses were then visualized in Discovery Studio Visualizer v. 19.1.0.18287 (Dassault Systèmes, Vélizy-Villacoublay, France). Since interactions with Caspase 8 showed the lowest binding energies, only results for this target were reported.

3. Results and Discussion

3.1. ABJ Treatments Inhibited Cell Viability and Reduced Proliferation of SW480 and SW620 Cells

Figure 1 shows the impact of ABJ on the cell viability of SW480 and SW620 cells. As observed, effects were stronger on SW620 cells, particularly for the 30% v/v ABJ dose, showing a −35.70% lower viability than SW480 cells for the same dose (Figure 1A). The calculated IC_{50} for both cell lines showed a lower ABJ dose needed to kill the same amount of cells.

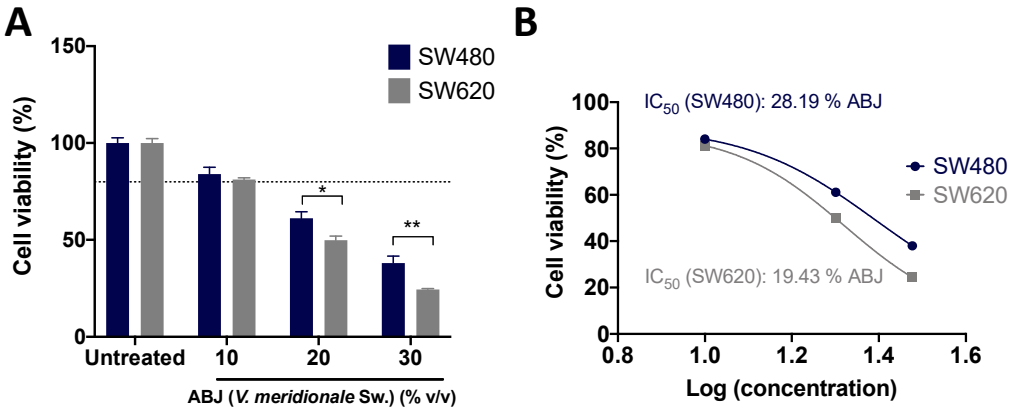

Figure 1. Impact of ABJ treatments on the cell viability of SW480 and SW620 cells. (**A**) Dose–response curves of cell viability from both cell lines after ABJ treatments; (**B**) calculated half-inhibitory concentrations (IC_{50}) from the cell viability tests. **ABJ:** Andean berry (*Vaccinium meridionale* Sw.) juice. The obtained IC_{50} values corresponded to 16.91 µg/mL and 11.66 µg/mL for SW480 and SW620, respectively. The asterisks represent significant differences in Tukey–Kramer's test ($p < 0.05$) between cell lines for each ABJ concentration. Untreated cells corresponded to DMEM + 3% FBS + ITS-treated cells.

Recently, our research group reported a similar IC_{50} concentration from SW480 cells (30.00 ± 0.11% v/v ABJ) [4]. Considering a previously reported polyphenolic composition from ABJ [3,4], the obtained IC_{50} doses corresponded to 26.30–38.15 µg 3,4-dihydroxybenzoic acid, 44.13–64.03 µg chlorogenic acid, 0.01–0.02 µg 2-hydroxycinnamic acid, 0.08–0.11 µg naringenin, 0.12–0.17 kaempferol, 128.19–185.98 µg gallic acid, 13.55–19.65

ellagic acid, 8.08–11.72 µg (+)-catechin, 21.36–30.99 µg rutin, 3.46–5.03 µg delphinidin chloride, and 13.56–19.67 µg cyanidin chloride. Although there are no reports from other research groups examining the impact of ABJ juice on colorectal cancer cell lines, the obtained IC_{50} are within reported experimental doses for anthocyanin-rich black raspberry (*Rubus occidentalis*) extracts (0.5–25 µg/mL) [10].

Figure 2 shows the antiproliferative effect of ABJ on SW480 (Figure 2A), SW620 (Figure 2B), and ABJ impact (10 and 30% *v*/*v*) on total Ki67 protein (Figure 2C). As observed, 20 and 30% ABJ (equivalents to 12 and 18 µg ABJ/mL) was the most antiproliferative treatment in SW480, whereas 20–30% *v*/*v* ABJ and 10–20% ABJ displayed the same trend for SW620. ABJ was more effective reducing Ki67 protein in SW620 than SW480 cells (Figure 2C). Except for our research group, who previously discovered the antiproliferative impact of four ABJ doses (3, 6, 8, and 10% *v*/*v*) on SW480 cells [11], there are no reports on the antiproliferative effect of ABJ on colorectal cancer cell lines. However, an aqueous methanolic cranberry (*Vaccinium macrocarpon* Ait.) inhibited up to ~35% of 24 h-SW620 cell viability but promoted SW480 growth, which could explain the results obtained at 48 h (10 and 20% ABJ) and 72 h (10% ABJ) for SW480 and SW620 cells, as berry-derived products contain macromolecules that potentially enhance proliferation [12]. Despite the fact that no reports were found for Ki-67 in vitro, administration of bilberry (*Vaccinium myrtillus*) dry extract reduced Ki67 in vivo, a well-known proliferation marker. Among the main phenolics from the extract, anthocyanins represented 36% of the extract, mainly composed of cyanidin-3-galactoside, delphinidin-3-galactoside, delphinidin-3-arabinoside, and cyanidin-3-glucoside [13].

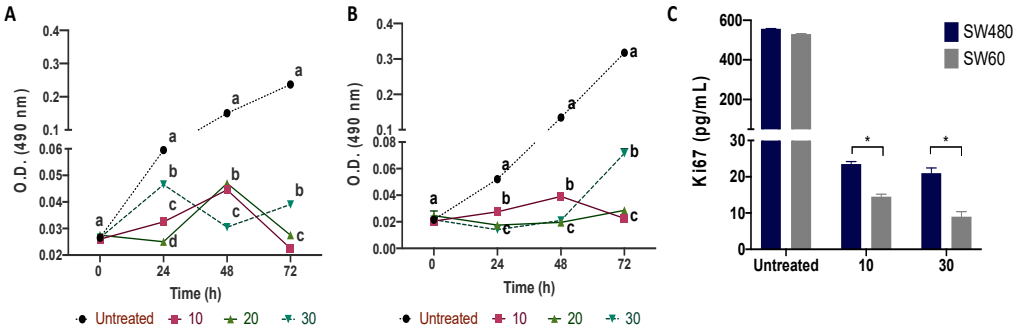

Figure 2. Antiproliferative effect of ABJ treatments on SW480 and SW620 cells. (**A**) Impact of ABJ treatments (10, 20, and 30% *v*/*v* ABJ) on SW480 cell proliferation, quantified as optical density (O. D.); (**B**) impact of ABJ treatments (10, 20, and 30% *v*/*v* ABJ) on SW620 cell proliferation, quantified as optical density (O. D.); (**C**) quantification of total Ki67 protein. Different letters in A and B represent significant differences ($p < 0.05$) in Tukey–Kramer's test between ABJ concentrations for each time measurement. The asterisks in C indicate significant differences ($p < 0.05$) in Tukey–Kramer's test.

3.2. Molecular Docking Analysis between Caspase 8 and Selected ABJ Anthocyanins

The evaluation of several pro-apoptotic markers against ABJ's phenolic compounds showed a major binding affinity between selected anthocyanins and caspase-8 (Figure 3) (ΔG peonidin-3-glucoside: −8.90 kcal/mol; ΔG cyanidin-3-galactoside: −8.80 kcal/mol; ΔG delphinidin-3-glucoside: −8.70 kcal/mol; ΔG cyanidin-3-glucoside: −8.60 kcal/mol). Results showed that van der Waals, conventional hydrogen bond, pi anion, and pi alkyl are the most common interactions between the selected phenolics and caspase 8, and the resulting energies are some of the highest reported between these phenolics and pro-apoptotic markers [14].

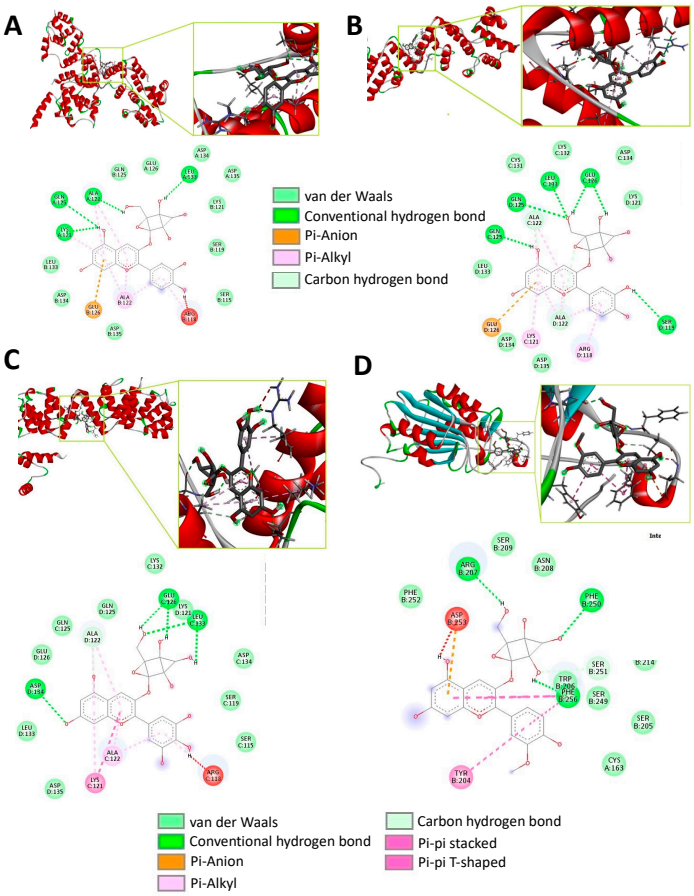

Figure 3. In silico analysis of the potential binding interactions between caspase 8 and (**A**) cyanidin-3-galactoside; (**B**) cyanidin-3-glucoside; (**C**) delphinidin-3-glucoside; and (**D**) peonidin-3-glucoside.

4. Conclusions

The results showed that ABJ inhibited cell viability and proliferation of SW480 and SW620 cells. As the impact was higher in SW620 compared to SW480, it could be suggested that a major influence of ABJ before a potential metastatic process results from potential interactions between ABJ phenolics and pro-apoptotic markers. Nonetheless, additional in vitro and in vivo studies are required to confirm these effects.

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