Andean Berry (*Vaccinium meridionale* Swartz) Juice Promotes Cytotoxic and Proapoptotic Effects in Human Early and Metastatic Colorectal Cancer Cells

Ivan Luzardo-Ocampo , Myriam Agudelo-Quintero , Sandra S. Arango-Varela * , Silvia A . Quijano * , Maria E. Maldonado-Celis , Jorge A. Lopera-Rodríguez

Posted Date: 30 March 2026

doi: 10.20944/preprints202603.2399.v1

Keywords: Andean Berry (*Vaccinium meridionale* Swartz) juice; apoptosis; colorectal cancer; antiproliferative activity; metastasis

 doi:10.20944/preprints202603.2399.v1

Article

Andean Berry (*Vaccinium Meridionale* Swartz) Juice Promotes Cytotoxic and Proapoptotic Effects in Human Early and Metastatic Colorectal Cancer Cells

Ivan Luzardo-Ocampo [1,2], Myriam Agudelo-Quintero [3], Sandra S. Arango-Varela [4,5,*], Silvia A. Quijano [6,*] , Maria E. Maldonado-Celis [7] and Jorge A. Lopera-Rodríguez [4,5]

[1] Tecnologico de Monterrey, Institute for Obesity Research, Ave. Eugenio Garza Sada 2501 Sur., Col.: Tecnológico, Monterrey, NL 64700, Mexico

[2] Tecnologico de Monterrey, School of Engineering and Sciences, Ave. Eugenio Garza Sada 2501 Sur., Col.: Tecnológico, Monterrey, NL 64700, Mexico

[3] Research and Graduate Program in Food Science, School of Chemistry, Universidad Autónoma de Querétaro, Cerro de las Campanas S/N, Qro. 76000, Mexico

[4] Grupo de Investigación e Innovación Biomédica, Facultad de Ciencias Exactas y Aplicadas, Instituto Tecnológico Metropolitano (ITM), Calle 73 No. 76A-354 Vía al Volador, Medellín, Antioquia 050012, Colombia

[5] Grupo de Investigación Biología Médica, Facultad de Ciencias Exactas y Aplicadas, Instituto Tecnológico Metropolitano (ITM), Calle 73 No. 76A-354 Vía al Volador, Medellín, Antioquia 050012, Colombia.

[6] Grupo de Investigación en Ecología y Conservación de la Biodiversidad (EcoBio), Facultad de Ciencias Básicas, Universidad Santiago de Cali, Cali 760001, Colombia

[7] Escuela de Nutrición y Dietética, Universidad de Antioquia, Carrera 75 No. 65-87, Medellín, Antioquia, Colombia

* Correspondence: sandraarango@itm.edu.co (S. S. A. -V.); silvia.quijano00@usc.edu.co (S. A. Q.)

Abstract

Andean berry (*Vaccinium meridionale* Swartz) is an underutilized fruit that could serve as a source of bioactive compounds with biological properties fostering apoptosis and cytotoxicity in colorectal cancer cells. This study aimed to evaluate the cytotoxic and proapoptotic effects of Andean berry juice (ABJ) in human SW480 and SW620 colon cancer cell lines, which are representative of early- and metastatic colorectal cancer. The juice was prepared from freeze-dried fruits, and several concentrations were assayed in cells. ABJ bioactive showed the strongest reductions in metabolic activity and proliferation observed in SW620 cells. ABJ treatments promoted early apoptosis while arresting the cell cycle in S phase (SW480) and in G2/M (SW620). Low mitochondrial depolarization was shown, but increased reactive oxygen species (ROS) accumulation was observed in both cell lines. More proteins involved in the apoptotic process were modulated in SW620 cells, whereas SW480 displayed greater fold changes in regulatory and stress-response proteins. Bioinformatics analysis highlighted predominantly extrinsic apoptosis in SW480 cells, while both extrinsic and intrinsic apoptosis were observed in SW620 cells. Results highlighted the cytotoxic and pro-apoptotic potential of the joint activity of ABJ polyphenolic compounds, demonstrating distinct mechanisms in early and metastatic cells *in vitro*.

Keywords: Andean Berry (*Vaccinium meridionale* Swartz) juice; apoptosis; colorectal cancer; antiproliferative activity; metastasis

1. Introduction

The development of cancer is a multi-stage process in which cells acquire traits such as uncontrolled growth, evasion of tumor suppressor signals, and resistance to cell death, ultimately

 doi:10.20944/preprints202603.2399.v1

reaching a state of replicative immortality that promotes their transformation into malignant cells. These traits are considered important targets for developing new cancer treatments [1]. Colorectal cancer (CRC) originates in the epithelium of the colon and/or rectum, and 90% of these cancers occur sporadically, without a family history, where the underlying causes are largely unknown [2]. According to a recent report, colorectal cancer is the 3rd most diagnosed cancer and the 2nd leading cause of cancer death, accounting for 1.9 million new cases and nearly ~1 million deaths in 2022 [3]. Other research estimates that by 2030, there will be a 60% rise in cases, reaching 2.2 million new diagnoses and 1.1 million deaths globally due to population aging [4,5].

The therapeutic management of CRC involves treatments such as chemotherapy, radiotherapy, and surgical removal of the tumor, among others [6]. Chemotherapeutic agents cause side effects, including the death of epithelial and liver cells, leading to symptoms such as fatigue, nausea, hair loss, and anemia [7]. These effects result from their cytotoxic activity against all cells in the body [8]. One approach that has been studied as a complement to chemotherapy is chemoprevention, which aims to harness the biological activity of phytochemicals found in foods and edible plants to prevent the development and progression of cancer [9]. Research in this field has shown that phytochemicals, such as phenolic compounds, can exert cytotoxic effects on cancer cells by interacting with molecules in cell signaling pathways associated with proliferation and by inducing cell death. [10].

One food type that has been evaluated for its chemopreventive properties is berries, which have generated particular interest in species of the *Vaccinium* genus. One such species, *Vaccinium meridionale* Swartz, commonly known as Andean berry, mortiño, or agraz, grows in Andean regions at elevations between 2000 and 3000 meters above sea level [11] and is used as an ingredient in processed products such as jams and juice. Several important compounds have been reported to be present in this species, including phenolic acids, derivatives of chlorogenic and caffeic acids, and several types of flavonoids, such as the anthocyanins cyanidin-3-glucoside, cyanidin-3-galactoside, and delphinidin 3-glucoside [12,13]. Previous studies have shown that most phenolic compounds in the juice are bioaccessible after gastrointestinal digestion [14,15]. Moreover, our research group has contributed to a comprehensive characterization of Andean berry juice using chromatographic techniques and has reported several polyphenolic compounds that agree with previous reports for this berry. In addition, aqueous extracts of this berry have displayed a decrease in antiproliferative activity in the colon adenocarcinoma cell line SW480 and its metastatic derivative SW620 [16]. The extract induced apoptosis in both SW480 and SW620 lines, with cell cycle arrest in the S, G2/M, and SubG0/G1 phases, respectively, without promoting mitochondrial membrane damage or oxidative stress.

Although previous reports on Andean berry in colon cancer cells have been published, none have explored the mechanistic pathways underlying its cytotoxic, antiproliferative, and pro-apoptotic effects. Hence, this research aimed to evaluate a widely consumed food matrix, specifically berry juice, for its ability to induce cell death in human colonic adenocarcinoma SW480 cells and their metastatic derivative, SW620, by performing a proteomic analysis and examining the mechanisms underlying the apoptotic process.

2. Results

2.1. Evaluation of the Cytotoxic Activity of Andean Berry Juice (ABJ) In Cell Lines

Figure 1 shows the effect of Andean berry (*Vaccinium meridionale* Swartz) juice (ABJ) on the metabolic activity (Figure 1A and Figure 1B), proliferation (Figure 1C and Figure 1D), levels of the Ki-67 protein (Figure 1E), granularity (Figure 1F), and the cloning efficiency (Figure 1G and Figure 1H) of SW480 and SW620 cells. All ABJ concentrations were cytotoxic when ABJ>12 mg/mL, with greater cytotoxicity in SW620 cells, as reflected by a lower IC_{50} value (-31.68 %) compared to SW480 cells (Figure 1B). As observed during the 72 h of ABJ treatments (Figure 1C-D), SW620 displayed markedly lower metabolic activity over time compared to SW480 cells. Although all ABJ treatments decreased Ki-67 levels in both cell lines, SW620 cells showed much lower values at 6 and 12 mg/mL

 doi:10.20944/preprints202603.2399.v1

(-95.06% and -91.81%, respectively) (Figure 1E). In contrast, granularity showed the opposite behavior (Figure 1F), with SW620 cells exhibiting a higher fold change than SW480 ($p < 0.05$). Similar trends were observed in the 24-plates for SW480 and SW620 cells in cloning efficiency, with no positive cells observed at any ABJ concentration (Figures 1G and 1H). These were quantified as absolute cloning efficiency (ACE) and relative cloning efficiency (RCE) in Supplementary Table A1.

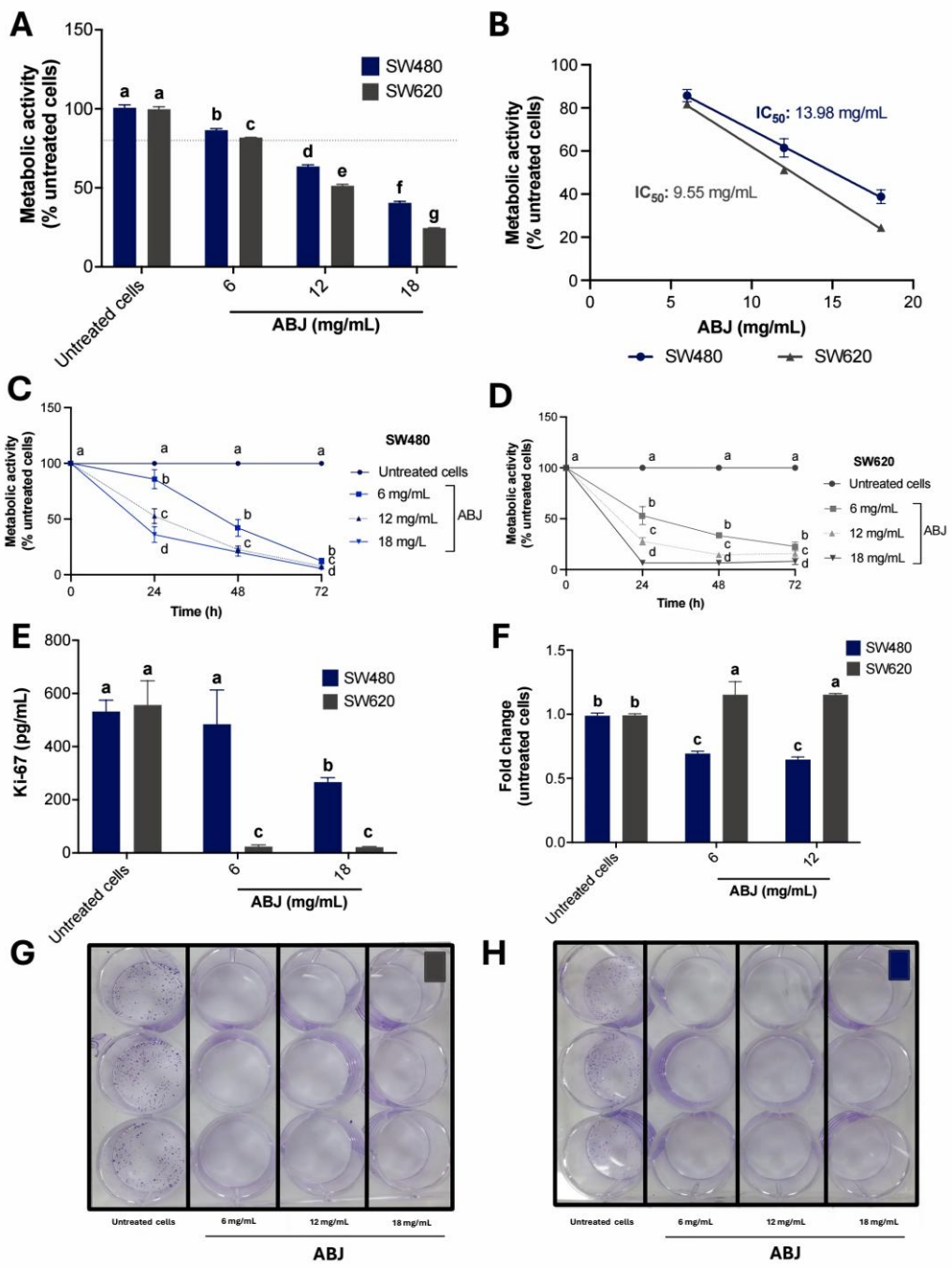


Figure 1. Effect of Andean berry (*Vaccinium meridionale* Swartz) juice (ABJ) on the metabolic activity, proliferation, and morphology of human SW480 and SW620 cells. Assessment of ABJ impact on (A) Metabolic activity; (B) Half-inhibitory concentration (IC_{50}) quantification; Antiproliferative effect on SW480 (C) and SW620 (D) cells; (E) Quantification of Ki-67 protein; (F) Cells' granularity; Evaluation of cloning efficiency of SW480 (G) and SW620 (H) cells. The results in A, C-F were expressed as the mean ± S.D. of at least two independent experiments in triplicate. Different letters express significant differences ($p<0.05$) by Tukey-Kramer's test. Assessments in A-D were conducted using the sulforhodamine B (SRB) assay. For the IC_{50} calculation in B a regression curve adjusted to biological models provided by GraphPad Prism v. 9.0 was used in the dose-

 doi:10.20944/preprints202603.2399.v1

response utility for the software. Untreated cells corresponded to either SW480 or SW620 cells in 2% FBS-DMEM. ABJ: Andean berry (*Vaccinium meridionale* Swartz) juice; IC_{50}: Half-inhibitory concentration; SW480: Human early colon cancer cells; SW620: Human metastatic colon cancer cells. Treatments in F were assayed at 6 and 12 mg/mL ABJ, since cells at 18 mg/mL showed severe signs of damage.

2.2. Impact of ABJ on Apoptosis and Cell Cycle Distribution of SW480 and SW620 Cells

Exposure of phosphatidylserine on the cell membrane is associated with apoptosis and can be detected by Annexin V staining. Sytox Green distinguishes apoptotic from necrotic cells. The representative dot chart (Figure 2A) shows the displacement of cells toward Q_2 (late apoptotic cells) and Q_3 (early apoptotic cells), as indicated by annexin V incorporation. Quantification of cell percentages in each quadrant (Figure 2B) showed the highest concentrations in Q4 and Q3, suggesting that ABJ treatments promote the transition from live to early apoptotic cells. Similar behavior was observed in both SW480 and SW620 cells, with the highest ABJ dose being the most pro-apoptotic in SW620.

The cell cycle is a sequence of four stages in which the cell duplicates its DNA and then divides. The quantification of cellular DNA content allows the cells treated for 24 hours to be distributed with ABJ concentrations (0, 6, and 12 mg/mL) in the G0/G1, S, and G2/M phases. Figure 2C shows representative histograms for the cell lines, and Figure 2D quantifies the number of positive cells at each cell cycle stage. It can be observed that SW480 and SW620 cells exhibit different responses to ABJ treatment. Metastatic cells tend to accumulate at the G0/G1 and G2/M stages, while SW480 cells gather at the S stage, indicating a cell cycle arrest in G0/G1 for SW480, which prevents preparation for DNA synthesis. In contrast, arresting the cycle in the S stage suggests triggering DNA duplication in SW620.

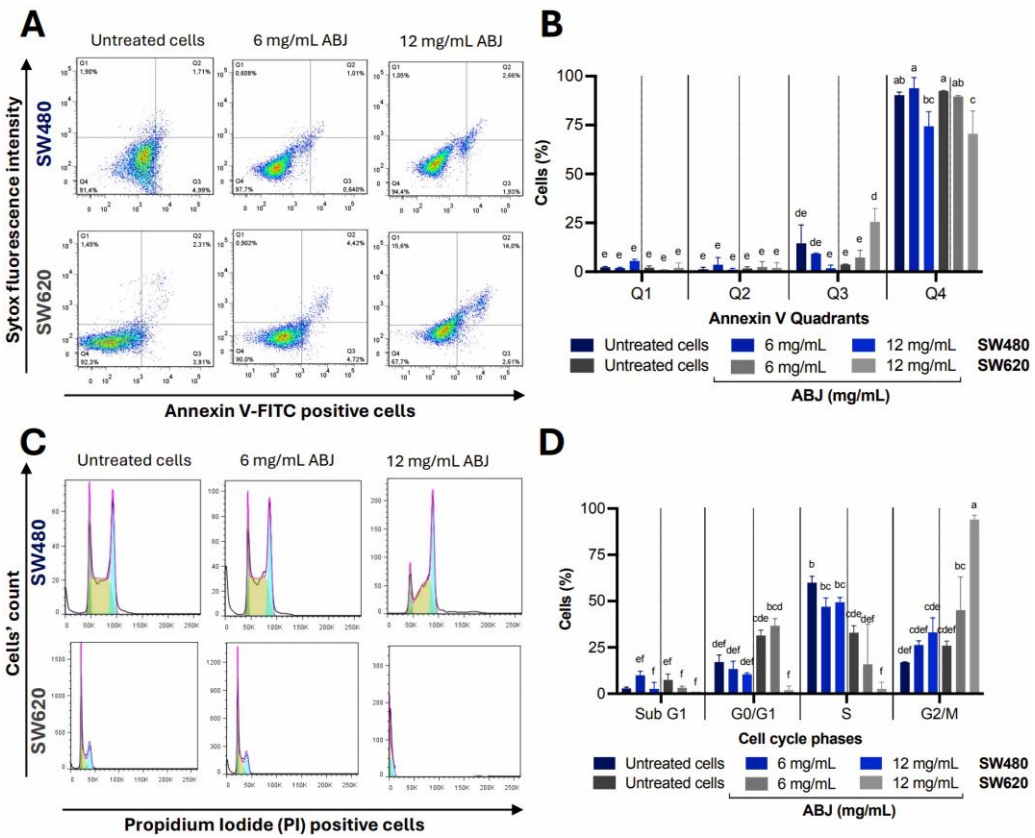


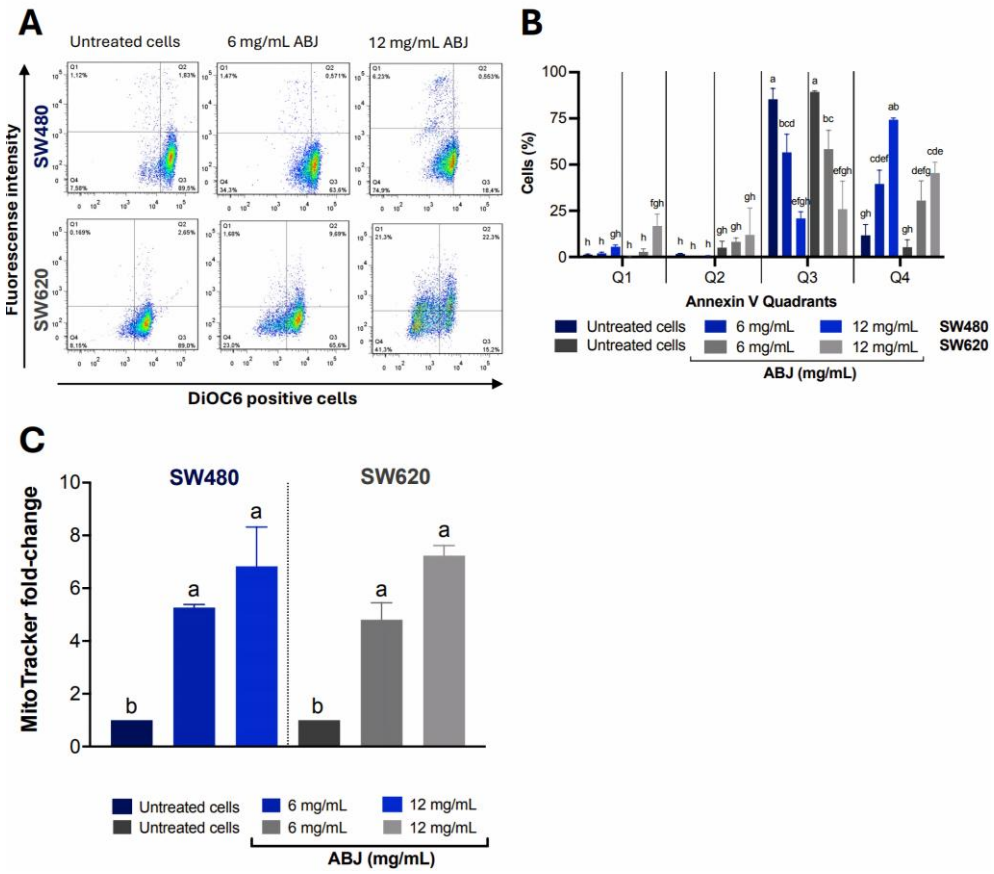
Figure 2. Evaluation of the impact of Andean berry (*Vaccinium meridionale* Swartz) juice (ABJ) on apoptosis and cell cycle distribution of SW480 and SW620 cells. Representative pictures of cells' plot by flow cytometry of the quadrants' distribution of cells as necrotic/apoptotic/live cells (A) and quantification of the percentage of cells

 doi:10.20944/preprints202603.2399.v1

on each quadrant (B). Evaluation of the cell cycle by propidium iodide (PI) staining showing representative pictures of the cell plots (C) and quantification of the number of cells (%) for each stage of the cell cycle (D). The results were expressed as the mean ± S.D. of at least two independent experiments in triplicate. Different letters express significant differences ($p<0.05$) by Tukey-Kramer's test. Untreated cells corresponded to either SW480 or SW620 cells in 2% FBS-DMEM. For the apoptosis assessment, Q1: necrotic cells; Q2: late apoptotic cells; Q3: early apoptotic cells; and Q4: live cells. For the cell cycle analysis (A), Sub G1: Cells with fragmented DNA before the G1 phase; G0/G1: Cell growth and preparation for DNA synthesis; S: Duplication of the cells' DNA; G2/M: Stage between protein synthesis for division and the mitotic stage. Untreated cells corresponded to either SW480 or SW620 cells without treatment (2 % FBS DMEM). ABJ; Andean berry (*Vaccinium meridionale* Swartz) juice; PI: Propidium iodide; SW480: Human early colon cancer cells; SW620: Human metastatic colon cancer cells.

2.3. Analysis of Mitochondrial Membrane Integrity and Activity in ABJ-Treated Cells

The reduction in mitochondrial membrane potential (ΔΨm) is an indicator of early apoptosis in response to treatment-induced damage. Staining with 3,3-dihexylocarbocyanine iodide (DiOC6) allows the mitochondrial response of the cells to the treatment to be evaluated. Double staining with propidium iodide (PI) allows the differentiation of live or dead cells with loss of mitochondrial membrane permeability. Figure 6A shows representative images of cells positive for DiOC6 staining, where a transition from Q3 to Q4 is observed as the ABJ concentration increases, as confirmed by the quantification of the percentage of cells (Figure 2B), where those quadrants displayed the highest accumulation of cells, with values being higher in SW480 than SW620. Results indicated that ABJ treatments promote a low membrane depolarization. Despite a discreet membrane polarization, results from MitoTracker staining of positive cells showed that all ABJ treatments significantly increased the number of stained cells ($p<0.05$) (Figure 3C), suggesting an increase in reactive oxygen species (ROS) with increasing ABJ concentrations.



 doi:10.20944/preprints202603.2399.v1

Figure 3. Impact of Andean berry (*Vaccinium meridionale* Swartz) juice (ABJ) on mitochondrial membrane integrity and activity in SW480 and SW620 cells. (A) Mitochondrial membrane potential (ΔΨm) representative pictures; (B) Quantification of the mitochondrial membrane potential by distribution of the cells on each quadrant; (C) MitoTracker fold-change quantification. The results were expressed as the mean ± S.D. of at least two independent experiments in triplicate. Different letters express significant differences ($p<0.05$) by Tukey-Kramer's test. Untreated cells corresponded to either SW480 or SW620 cells in 2% FBS-DMEM. Q1 (DiOC6−/PI+): dying cells (nonapoptotic/necrotic), low ΔΨm, and low membrane integrity; Q2 (DiOC6+/PI−): Late apoptosis, high ΔΨm, and low membrane integrity; Q3 (DiOC6+/PI−): High ΔΨm and good membrane integrity; Q4 (DiOC6−/PI−): low ΔΨm, and good membrane integrity. ABJ: Andean Berry (*Vaccinium meridionale* Swartz) juice; DiOC6: 3,3′-dihexyloxacarbocyanine iodide; PI: propidium iodide; SW480: Human early colon cancer cells; SW620: Human metastatic colon cancer cells.

2.4. Human Apoptosis Proteome Analysis in ABJ-Treated Cells

The expression of proteins associated with apoptosis (Figure 4) was evaluated in SW480 and SW620 cells treated with the IC_{50} concentration of ABJ for each cell line, as determined by the results in Figure 1B. The relative fold change in expression was determined relative to untreated cells. To facilitate visualization of the assayed proteins, all were clustered into three groups: pro-apoptotic proteins and caspases (Figure 4A-B), anti-apoptotic proteins (Figure 4C-D), and proteins related to regulation and stress response. Overall, SW620 cells showed more proteins that varied significantly between the untreated and treated cells, but fold-changes were lower than those observed for SW480 proteins. Among the pro-apoptotic proteins and caspases (Figure 4A and Figure 4B), phospho-p53 (S392) and SMAC showed similar behavior in both cell lines. In contrast, procaspase-3, cytochrome C, Bcl-2-associated death promoter (Bad), Bcl-2-associated X protein (Bax), death receptor 4 (DR4), and death receptor 5 (DR5) showed opposite trends, indicating different pro-apoptotic mechanisms in both cell lines. In the anti-apoptotic protein cluster (Figure 4C and 4D), SW620 showed decreased expression of more anti-apoptotic proteins, such as Survivin, X-linked inhibitor of apoptosis (XIAP), and B-cell lymphoma extra-large protein (Bcl-xL), indicating different IC_{50} ABJ behavior in these cells. For proteins related to regulation and stress response, ABJ treatment increased p27, p21, Paraoxonase-2 (PON2), Clusterin, heat-shock protein 27 (HSP27), and Claspin in SW480 cells, whereas the opposite trend was observed in SW620 cells.

 doi:10.20944/preprints202603.2399.v1

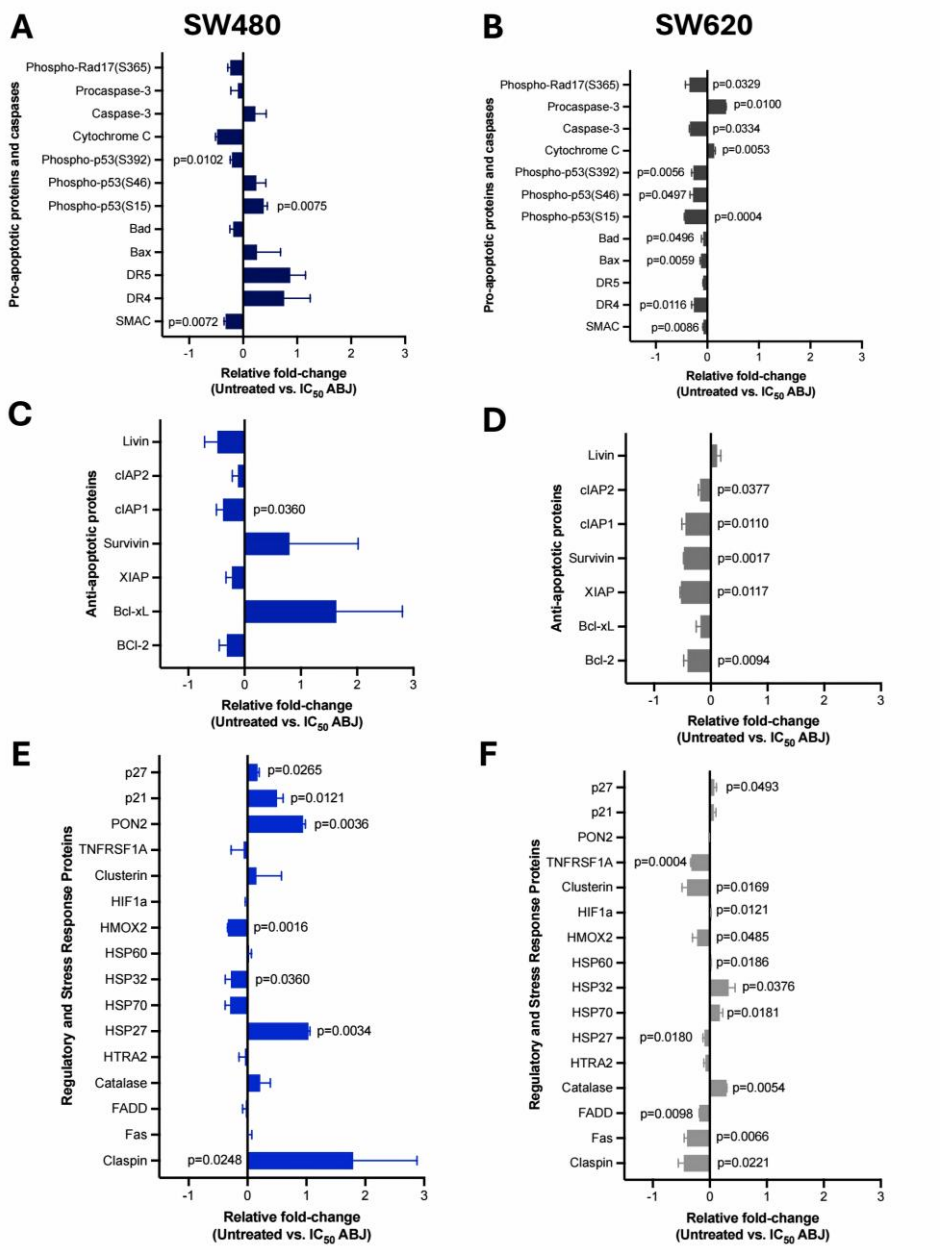


Figure 4. Assessment of Andean berry (*Vaccinium meridionale* Swartz) juice effect on the relative fold-change of assessed cytokines from the apoptotic process in SW480 and SW620 cells. Cytokines were distributed into pro-apoptotic proteins and caspases (A, B), anti-apoptotic proteins (C, D), and regulatory and stress-response proteins (D, E). The results were expressed as the mean ± S.D. of at least two independent experiments in triplicate. Only significant p-values were indicated in the figures, assessed by Student's t-test. The significance was assessed by comparing the cytokine's relative intensity between the untreated (2% FBS DMEM-only) cells and those treated with the half-inhibitory concentration of Andean berry (*Vaccinium meridionale* Swartz) juice (IC_{50} ABJ). Bad: Bcl-2-associated death promoter; Bax: Bcl-2 associated X protein; BCl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra-large; cIAP1: Cellular inhibitor of apoptosis protein 1; cIAP2: Cellular inhibitor or apoptosis protein 2; DR4: Death receptor 4; DR5: Death receptor 5; FADD: Fas-associated death domain protein; Fas (CD95): FS-7 associated protein; HIF1a: Hypoxia-inducible factor 1, alpha subunit; HMOX2: Heme oxygenase 2; HSP27: Heat-shock protein 27; HSP32: heat-shock protein 32; HSP60: Heat-shock protein 60; HSP70: Heat-shock protein 70; HTRA: High-temperature requirement A; PON2: Paraoxonase 2; SMAC: Second mitochondria-derived activator of caspase; SW480: Human early colon cancer cells; SW620: Human metastatic colon cancer cells; TNFRSF1A: Tumor necrosis factor receptor superfamily, member 1A; XIAP: X-linked inhibitor of apoptosis protein.

 doi:10.20944/preprints202603.2399.v1

A bioinformatic analysis was conducted on the STRING platform (Figure 5) for the assessed proteins, showing that the main biological process enrichment (Figure 5A and Figure 5B) was the apoptotic process of dendritic cells and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling pathway, while both extrinsic and intrinsic apoptotic mechanisms were highlighted for the SW620 cells. Regarding Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, apoptosis, the p53 signaling pathway, and necroptosis are among the highest-ranked pathways based on false discovery rate (FDR) and the program's calculated strength (Figure 5C and 5D).

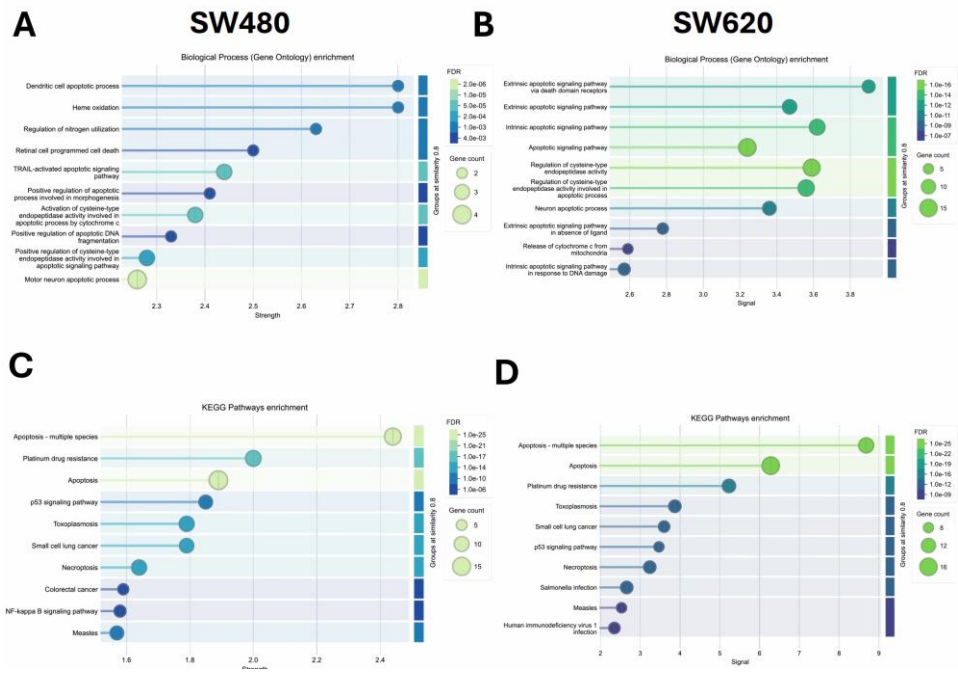


Figure 5. Assessment of Andean berry (*Vaccinium meridionale* Swartz) juice effect on the relative fold-change of assessed cytokines from the apoptotic process in SW480 and SW620 cells. Cytokines were distributed into pro-apoptotic proteins and caspases (A, B), anti-apoptotic proteins (C, D), and regulatory and stress-response proteins (D, E). The results were expressed as the mean ± S.D. of at least two independent experiments in triplicate. Only significant p-values were indicated in the figures, assessed by Student's t-test. The significance was assessed by comparing the cytokine's relative intensity between the untreated (2% FBS DMEM-only) cells and those treated with the half-inhibitory concentration of Andean berry (*Vaccinium meridionale* Swartz) juice (IC50 ABJ). Bad: Bcl-2-associated death promoter; Bax: Bcl-2 associated X protein; BCl-2: B-cell lymphoma 2; Bcl-xL: B-cell lymphoma-extra-large; cIAP1: Cellular inhibitor of apoptosis protein 1; cIAP2: Cellular inhibitor or apoptosis protein 2; DR4: Death receptor 4; DR5: Death receptor 5; FADD: Fas-associated death domain protein; Fas (CD95): FS-7 associated protein; HIF1a: Hypoxia-inducible factor 1, alpha subunit; HMOX2: Heme oxygenase 2; HSP27: Heat-shock protein 27; HSP32: heat-shock protein 32; HSP60: Heat-shock protein 60; HSP70: Heat-shock protein 70; HTRA: High-temperature requirement A; PON2: Paraoxonase 2; SMAC: Second mitochondria-derived activator of caspase; SW480: Human early colon cancer cells; SW620: Human metastatic colon cancer cells; TNFRSF1A: Tumor necrosis factor receptor superfamily, member 1A; XIAP: X-linked inhibitor of apoptosis protein.

2.5. Principal Components Analysis (PCA) of the Observed Biological Processes Modulated by ABJ

Results from the PCA analysis (Figure 6) showed that only 2 components explained >90 % of the total variation (Figure 6A). Two differentiated clusters were observed for SW480 and SW620 cells (Figure 6B), where the ABJ treatment on SW480 cells mostly impacted the distribution of cells in apoptosis (Q2-Q4), three quadrants of the DiOC6 staining (DQ1-DQ3), granularity, metabolic activity, and two stages of the cell cycle (SubG1 and S). On the other hand, ABJ treatment primarily modulated the Q3 quadrant of apoptosis, the G0/G1 and G2/M stages of the cell cycle, and the D-Q4 stage, as indicated by DiOC6, Ki-67, and MitoTracker staining in SW620 cells. Principal component 1

 doi:10.20944/preprints202603.2399.v1

(PC1) variation was primarily driven by Ki-67, Q2 of the apoptosis process, and D-Q2 from DiOC6 staining in the positive direction, whereas tumor necrosis factor receptor superfamily member 1A (TNFRSF1A), Clusterin, and heat-shock protein 70 were in the negative direction. For PC2, Bax, Bad, and procaspase proteins explained their positive variation, and the Q3 quadrant of apoptosis, G2/M and G0/G1 stages of the cell cycle, and the D-Q4 quadrant from DiOC6 staining explained their negative variation (Supplementary Table S2).

A

PC	Percentage	Cumulative Percentage
1	65.314	65.31
2	32.820	98.13
3	1.838	99.97
4	0.028	100.00
5	0.000	100.00

B

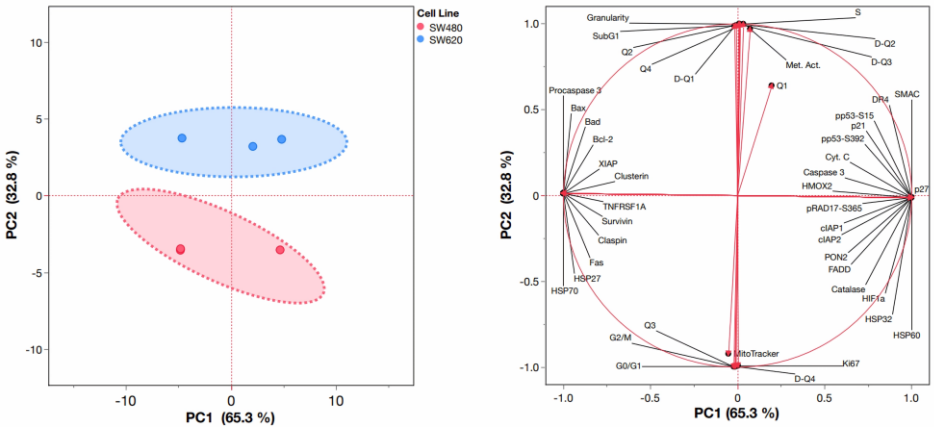


Figure 6. Principal components analysis (PCA) for the assessed variables impacted by Andean berry (*Vaccinium meridionale* Swartz) juice on SW480 and SW620 cells. (A) Individual and cumulative percentages covered by each principal component (PC); (B) Scatter plot and loading plots for the first and the second component after the evaluation of all variables.

3. Discussion

This study demonstrated differential programmed death induction (apoptosis) in an *in vitro* model of colorectal adenocarcinoma (CRC), using the SW480 cell line and its SW620 metastatic derivatives, treated with Andean berry juice (ABJ) (*Vaccinium meridionale* Swartz). The results showed that ABJ significantly decreased cell viability when cells were exposed to different concentrations of the juice for 24 hours. The IC_{50} values reported in this study are close to those reported in other studies that evaluated different concentrations of ABJ in SW480 and SW620 cells [17,18]. In other studies evaluating cytotoxicity using the same cell lines, the metastatic cells showed greater sensitivity to the juice, with IC_{50} values lower than those reported for SW480 in this investigation [19]. Other products, such as vinegar, nectar, and even green and black tea obtained from the leaves of this plant species, have been shown to reduce cellular metabolic activity and promote antiproliferative activity in these cell lines [20,21]. Similar results have also been obtained in the combination of ABJ with Aspirin, a well-known anti-inflammatory drug [18,22]. The cytotoxic, antiproliferative, and antitumor potential of *V meridionale* has also been evaluated in *in vitro* models of various cancers, including fibrosarcoma (HT1080) cells, transformed leukemia cells (MOLT4 cell line), and HT29 colorectal adenocarcinoma cells [14,23,24]. Additionally, the antitumor effect of ABJ has been demonstrated by a drastic, significant decrease in cell cloning capacity reported in other studies using SW480 cells [22]. The results of this study show the potential chemopreventive effects of this fruit against various cancers.

The Ki-67 protein is a marker of cell proliferation, and an increase in its concentration is associated with greater proliferative capacity [25]. Together with p53 expression, it is related to early

 doi:10.20944/preprints202603.2399.v1

relapse and distant metastasis in colorectal cancer patients [26]. Results from the amount of protein found in cells after ABJ treatment indicated that SW620 cells are more prone to accumulate Ki-67, consistent with previous reports of its primary association with metastatic cells and excessive cell proliferation in colorectal cancer, yet ABJ showed limited ability to reduce proliferation in this cell line. Moreover, ABJ failed to reduce cell size or granularity in SW620 cells. However, these cells are readily misclassified as granulocytes due to their size and high variability in optical path delay when examined by flow cytometry [28].

When evaluating a chemopreventive agent, it is important to determine its capacity to modulate the cell cycle, as assessing both apoptosis and the cell cycle provides information about cancer molecular pathogenesis and how tumor cells respond to therapy. In SW480 cells, the percentage of cells in S phase decreased, then increased in G2/M phase as ABJ concentration increased. These results are consistent with those reported in a previous study evaluating ABJ, in which an increase in the percentage of SW480 cells in the G2/M phase was observed at an ABJ concentration of 18 mg/mL. It was proposed that ABJ can modulate mitotic division [16]. On the other hand, in SW620 cells, the percentage of cells in the G2/M phase decreased, whereas that in the S phase increased significantly; this is consistent with their expected behaviour in metastatic cells, where proliferative capacity is increased [30]. Based on this, it can be stated that treatment of SW620 cells with ABJ modulates the continuity of the cycle towards G2/M.

The induction of apoptosis is one of the expected effects of a chemopreventive agent. In the present work, the ability of ABJ to induce apoptosis in the two cell lines was evaluated to compare the responses of cells that share a monoclonal origin but exhibit significant phenotypic differences, as SW620 cells were originally derived from a lymph node metastasis of the same tumor that gave origin to SW480 cells [31]. The Annexin V and Sytox Green assays showed that ABJ induces phosphatidylserine exposure without membrane depolarization. These results are consistent with those reported in other studies [16,18], in which the evaluated concentrations of an aqueous extract of the Andean berry induced apoptosis without mitochondrial damage in the two cell lines, but increased reactive oxygen species [32]. A comprehensive evaluation of the effect of procyanidins from different berries, such as wild lowbush berry (*Vaccinium myrtillus*), highbush blueberry (*Vaccinium corymbosum*), lingonberry (*Vaccinium vitis-idaea*), raspberry (*Rubus idaeus*), wild blackberry (*Rubus fruticosus*), thornfree blackberry (*Rubus fruticosus* 'Thornfree'), redcurrant (*Ribes rubrum*), gooseberry (*Ribes uva-crispa*), blackcurrant (*Ribes nigrum*), jostaberry (*Ribes nidigrolaria*), and cranberry (*Vaccinium macrocarpon*) showed apoptosis induction in SW480 and SW620 cells in a more potent manner than apple procyanidins. Still, these compounds did not sensitize either cell line to TRAIL-induced apoptosis [33]. Ellagic acid, one of the phenolic compounds identified in ABJ, has been linked to chemosensitivity effects on human colorectal carcinoma cells. Its evaluation in HT-29 and SW480 in a 2.5-25 μg/mL (which is much lower than the reported values for ABJ) decreased the proliferation of these cells, enhanced the Bax: Bcl-2 ratio, triggered caspase-3 activation, and promoted apoptosis in these cells, making them more susceptible to the treatment with well-known chemotherapy agents like 5-fluorouracil [34]. Gallic acid, also present in ABJ, prevents cellular proliferation and arrests the cell cycle in G0/G1 by decreasing cyclin D1 level, results that have been observed in human Caco-2 and HCT-15 cells, also cells from primary tumor, low metastatic potential, and exhibiting mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) protein like SW480 cells [35,36]. More recently, chlorogenic acid concentrations up to 2000 μM decreased the G1 phase and increased the subG1 phase in SW480 cells, suggesting apoptosis induction, as fragmented DNA was detected by the authors [37].

An additional ability of different ABJ concentrations to induce cell death was evaluated by staining for outer and mitochondrial membranes, assessing expression of markers associated with apoptotic signaling pathways, and visualizing apoptotic cell nuclei. MitoTracker dyes have been found to be released from mitochondria, like other dependent dyes, and this release could be affected by the ROS levels in the cells [38], confirming the need for additional assays evaluating mitochondrial potential and integrity, such as the DiOC6 staining conducted in this research. Previously, no

 doi:10.20944/preprints202603.2399.v1

mitochondrial damage was reported in ABJ-treated SW6480 and SW620 cells at ABJ concentrations higher than those used in this research, and ABJ did not induce ROS production in the cells [16]. However, additional mitochondrial mechanisms were observed following MitoTracker analysis, with marked mitochondrial damage evident after ABJ treatments. In SW480 and HT-29 cells, chlorogenic acid has induced marked mitochondrial ROS production, up to 3-fold, at concentrations ranging from 0 to 2000 μM, thereby increasing activation of proapoptotic molecules, such as caspase-3 [37].

The evaluation of proteomic profiles of SW480 and SW620 cells treated with ABJ allows us to verify the Annexin V assay results, as the expression of proteins involved in the apoptotic process was assessed. The results obtained show the overexpression of pro-apoptotic markers of the extrinsic pathway, such as caspase 3, cIAP-2, TRAIL/DR4, and DR5 in SW480 cells, in addition to the activation of cell cycle modulating proteins, such as claspin and the cyclin-dependent kinase inhibitor 1A (CDKN1A), and response markers against cellular stress, such as catalase, PON2, HSP27, and HSP60. The activity of all these markers confirms the results obtained in the Annexin V, DiOC6, and MitoTracker assays, as does the increased expression of the extrinsic apoptosis complex TRAIL-DR4. These results enable the formulation of hypotheses about the response of adenocarcinoma cells to a chemopreventive agent. Previous research from our group [22] showed overexpression of TRAIL-DR4, TRAIL-DR5, PON2, and CDKN1A proteins in SW480 cells treated with 30% v/v ABJ and Aspirin, underexpression of catalase, and an increase in caspase3 expression at 12 mg/mL ABJ. Regarding SW620 cells, the expression of these apoptosis-determining markers was observed through the overexpression of the non-active form of caspase 3 (extrinsic apoptosis) and the release of cytochrome c (intrinsic apoptosis), in addition to the underexpression of anti-apoptotic markers such as Bcl-2 and XIAP. The relationship to specific molecular pathways linked to apoptosis was confirmed through bioinformatics enrichment analysis, which also supported most of these conclusions, drawn from studies with berries and molecular markers of apoptosis; however, a proteomics comparison between SW480 and SW620 is novel. The differential expression of these proteins allows a differential molecular response to be observed between the two cell lines treated with ABJ at 6 and 12 mg/mL. This needs to be investigated at a deeper level by inhibiting specific markers, allowing the ability of ABJ to induce apoptosis through intrinsic or extrinsic pathways to be distinguished. *In silico* analysis predicting the impact of specific compounds on protein markers of apoptosis provides insights into molecular interactions between phytochemicals. Predicting the molecular interaction between apoptosis markers and the phytochemicals present in the Andean berry, such as chlorogenic acid, caffeic acid, and cyanidin 3 glucoside [39,40], could also explain the effect of ABJ on the activation or inhibition of the proteins of interest to be characterized. Previous findings have shown that some of the ABJ compounds evaluated exhibit affinity for proteins involved in apoptosis, including Bcl-2, cytochrome c, and caspase-3. Particularly, cyanidin-3-glucoside, which is present in high concentrations in the berry, exhibits elevated coupling affinity for receptors such as TRAIL-DR4, which, when activated, triggers caspase activity that executes apoptosis [41].

The PCA analysis aimed to integrate the results and provide greater discrimination between SW480 and SW620 cells, which were also applied to selected cell lines, linking to the composition of the molecular entities used to challenge the cells. The dimensional reduction of components linked to SW480 cells is more closely associated with cell cycle progression, metabolic activity, and early cell cycle stages. In contrast, SW620 cells were associated with intermediate stages of the cell cycle, as indicated by Ki-67 and MitoTracker. Results from this research and their analysis have extensively linked SW620 cells, as metastatic cells, with increased proliferation, but phenolic compounds (anthocyanins, proanthocyanidins, and flavonols, among others), either as isolated compounds or jointly acting from complex extracts of berries, can strongly inhibit SW620 proliferation, promote apoptosis, and decrease anti-inflammatory cytokines linked to the nuclear factor kappa B (NF-κB) pathway [43].

4. Materials and Methods

 doi:10.20944/preprints202603.2399.v1

2.1. Preparation of Juice From Freeze-Dried Andean Berry (Vaccinium Meridionale Swartz) Juice (ABJ)

A very well-standardized Andean berry juice (ABJ) was prepared following previously reported methodologies [15]. This involved carrying out chemical characterization of the juice, in which the total phenolic compounds (3570 ± 260 mg gallic acid equivalents/100 mL), total flavonoids (2310 ± 20 mg of (+)-catechin equivalents/100 mL), and total monomeric anthocyanins (129 ± 20 mg cyanidin-3-O-glucoside/100 mL). The individual phenolic compounds, identified by high-performance liquid chromatography coupled to diode-array detection (HPLC-DAD), included gallic acid (658.45 ± 8.80 µg/g), chlorogenic acid (35.31 ± 2.90 µg/g), caffeic acid (11.52 ± 0.10 µg/g), ellagic acid (69.31 ± 5.00 µg/g), *p*-coumaric acid (6.50 ± 0.20 µg/g); 3,4-dihydroxybenzoic acid (809.23 ± 0.87 µg/g); 2-hydroxycinnamic acid (0.38 ± 0.01 µg/g), (+)-catechin (41.32 ± 0.10 µg/g), rutin (111.23 ± 0.30 µg/g), morin (133.88 ± 7.70 µg/g), and kaempferol (14.57 ± 0.10 µg/g) [22]. Novel analysis of some of these compounds found in the juice are presented in Supplementary Table S3 and Supplementary Fig. S1

Furthermore, the juice exhibited antioxidant capacity *in vitro*, as demonstrated by ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition values of 127.60 and 35.43 µmol Trolox equivalents/mL, respectively [16].

2.2. Cell culture

Human SW-480 colorectal adenocarcinoma cells [SW-480] (ATCC CCL-228) and their metastatic derivatives SW-620 [SW-620] (ATCC CCL-227) were obtained from American Type Culture Collection (ATCC). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) high-glucose (25 mM), supplemented with L-glutamine, fetal bovine serum (FBS, 10% v/v), and antibiotics (100X, 1% v/v). Unless indicated otherwise, all reagents used for the cell culture maintenance were acquired from Gibco (Thermo Fisher Scientific, Waltham, MA, US). Cells were maintained in a humidified 5 % CO_2 atmosphere at 37 °C.

2.2.1. Assessment of the Metabolic Activity of the Cells Exposed to Andean Berry (*Vaccinium meridionale* Swartz) Juice (ABJ)

From both lines, 2×10^4 cells/well were cultured in 96-well plates. After 24 hours, different concentrations of ABJ (0, 6, 12, and 18 mg/mL) were added. After the exposure time had elapsed, the cultures were interrupted by adding 50 µL of 50% trichloroacetic acid. They were incubated at 4 °C for 1 h, then the acid was replaced with sulforhodamine B (SRB) (0.4% w/v, diluted in 1% v/v acetic acid) ($C_{27}H_{29}N_2NaO_7S_2$ powder, BioReagent, Sigma-Aldrich, St. Louis, MO, US) for 30 min. Subsequently, the SRB was stirred together with 1% v/v acetic acid. For measurement, SRB was solubilized in 200 µL of Tris-HCl buffer (10 mM, pH 10.5) for 20 min. Optical density was measured at 490 nm using a Varioskan plate reader (Thermo Fisher Scientific, Waltham, MA, US). Five replicates per treatment were performed, and the mean inhibitory concentration (IC_{50}) was determined using a statistical regression model. The impact on the metabolic activity was assessed as follows: metabolic activity (%): [(Absorbance sample/Absorbance of negative control]×100 %, where the negative control corresponded to untreated cells (cells cultured in DMEM with 2 % FBS).

2.2.2. Impact of Andean Berry (*Vaccinium meridionale* Swartz) Juice on Cell Proliferation

The sulforhodamine B (SRB) assay was used. Both lines were cultured at 3×10^3 cells/well. After 24 h, they were incubated with ABJ (0, 6, 12, and 18 mg/mL) for 0, 24, 48, and 72 hours. For the 72-h culture, the medium was reconstituted every 48 h with the respective concentrations. For staining, the process described above was carried out. Five replicates of each treatment were performed, and curves of the cells' growth over time were plotted. Untreated cells (2 % FBS-DMEM) were used as a control.

2.2.3. Evaluation of the Expression of the Ki-67 Protein

 doi:10.20944/preprints202603.2399.v1

For this assay, 1.5×10^6 cells/mL from both cell lines were seeded and incubated at 37°C and 5% CO_2. After 24 h, the medium was replaced by the 6 and 18 mg/mL juice treatments. Subsequently, the cells were incubated under the same conditions for 24 h, the supernatant was removed, and the cells were treated according to the manufacturer's instructions (Human Ki67/MKI67 DuoSet ELISA, R&D Systems Inc., Minneapolis, MN, United States). The optical density at 450 nm was then measured in the Varioskan plate reader (Thermo Fisher). The Ki-67 concentration was calculated using the manufacturer-standardized curve.

2.2.4. Assessment of *V. meridionale* Swartz juice On the Cells' Granularity

Cell cultures of both lines were treated with ABJ (0, 6, 12, and 18 mg/mL) for 24 h and subsequently observed under a microscope at 40X to assess changes in confluency and cell size. Additionally, the average fluorescence intensity of the parameters was recorded by flow cytometry: SSC (side scatter) for cell granularity. The results were expressed as fold-change relative to the control (untreated cells, growth in 2 % FBS-DMEM).

2.2.5. Evaluation of the Effect of Andean Berry (*Vaccinium meridionale* Swartz) Juice (AB J) on Cloning Efficiency

The cells (2.50×10^2) were seeded in 1 mL of maintenance medium (10% FBS DMEM) and incubated for 24 h. The cells were then treated with ABJ (0, 6, 12, and 18 mg/mL) with 3 replicates per treatment and incubated for 24 h. Subsequently, the treatments were replaced with maintenance medium and incubated for 7 days, after which the medium was changed. The adherent cells were fixed with a Carnoy solution (methanol and acetic acid in a 3:1 ratio) and stained with crystal violet (0.5% w/v) for 1 h. Following this, the dye was removed and washed with distilled water, the plates were left for 24 h, and colony counting was performed under the microscope with the inclusion criterion (1 colony, 30 cells or more). The absolute cloning efficiency (ACE) was calculated as the percentage of colonies relative to the number of cells seeded, while the relative cloning efficiency (RCE) was calculated as the percentage of $ACE_{treatments}$ relative to $ACE_{control}$.

2.2.6. Analysis of Apoptosis by Sytox/Annexin V Staining

SW480 and SW620 cells were seeded at 1×10^6 cells/mL and incubated for 24 hours. After the period, the medium was replaced with the ABJ treatments (0, 6, and 12 mg/mL), and the cultures were incubated for 24 h. After trypsinization, the precipitate was stained with 1 µg SYTOX™ Green (S7020, Invitrogen, Thermo Scientific) and Annexin-V-FITC 1X (Annexin V-FITC Apoptosis Detection Kit, APOAF-20TST, Sigma-Aldrich). Fluorescence intensity was measured by flow cytometry, with 10000 events recorded.

2.2.7. Effect of the Juice on the Cell Cycle

Cell cycle analysis was performed by staining cellular DNA with propidium iodide (PI). Both cell lines were seeded at a concentration of 1×10^6 cells/mL in maintenance medium and incubated for 24 h at 37°C and 5% CO_2. After the adherence period, they were treated with ABJ (0, 6, and 12 mg/mL) and incubated for 24 h. Subsequently, they were treated with 500 µL of 1X trypsin-EDTA (Sigma-Aldrich). The suspension was centrifuged (1500× g, 10 min), and the precipitate was fixed with 2 mL of cold 70% v/v ethanol and preserved at 4 °C for 24 h. Finally, the ethanol was removed, and after the last wash, the precipitate was reconstituted in 500 µL of 1× PBS. A mixture of 1 µL PI and RNAase (2 mg/mL) was added, followed by incubation at room temperature for 30 min. The suspensions obtained were analyzed using the FACSCantoII Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, US), analyzing 10000 cell events/min.

2.2.8. Analysis of Mitochondrial Membrane Permeabilization By Staining With 3,3-Dihexylocarbocyanine Iodide (DiOC6) and Propidium Iodide (PI)

 doi:10.20944/preprints202603.2399.v1

A cell concentration of 1×10^6 cells/mL of the two cell lines was treated with concentrations of ABJ (0, 6, and 12 mg/mL) for 24 h. Subsequently, all cells were collected by PBS and trypsin washes, stained with 500 nM DiOC6 and PI (2.5 µg/mL), and incubated at room temperature (25 ± 1 °C) for 15 min. Cellular events (10000/min) were recorded to quantify the number of cells positive for DIOC6/PI staining.

2.2.9. Analysis of Apoptosis by Staining with MitoTrackerTM Red CMXRos

SW480 and SW620 cells were seeded at a concentration of 1×10^6 cells/mL and treated with ABJ concentrations (0, 6, and 12 mg/mL) for 24 h. Then, the cells were collected after washing with PBS, trypsinized, and centrifuged at 1500× g for 4 min. The precipitate obtained was supplemented with MitoTrackerTM Red CMXRos (ThermoFisher) (150 nM) and incubated at 37 °C for 30 min. After this, the coloring was removed by 3 washes with PBS. The precipitate was resuspended in 250 µL of PBS, and the fluorescence was measured by flow cytometry at 10000 cell events/min.

2.2.10. Analysis of the Modulation of Proteins

SW480 and SW620 cells were seeded at a concentration of 1×10^6 cells/well in culture dishes and incubated for 24 h at 37°C. Subsequently, the cells were treated with 12 mg/mL of the juice for 24 h. After the incubation period, the cells were lysed using Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) (190 µL, dissolved in 9.81mL of the protein inhibitor) in lysis buffer for 30 min at 4 °C. Then, protein concentration was quantified using the Bicinchoninic acid (BCA) kit (PierceTM Thermo Fisher). For each membrane of the Proteome Profiler Human Apoptosis Array kit (R&D Systems, Minneapolis, MN, USA), the same amount of total protein lysate (400 µg) was added, following the manufacturer's instructions. The images were then quantified using the ChemiDoc XRS+ kit (Bio-Rad, Hercules, CA, US), and the expression intensity was determined using the Decodon Delta 2D software (Decodon, Greifswald, Germany). The results were expressed as fold change (FC) relative to the intensity of each protein in its respective control.

For the bioinformatics assessment, evaluations of associated biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed using the STRING (https://string-db.org/, accessed on 1st December, 2025) protein-protein interaction networks and functional enrichment analysis, based on the obtained fold-changes of proteins related to the apoptotic process [44].

2.3. Statistical Analysis

Unless indicated, the results were expressed, when appropriate, as the mean ± S.D. of at least two independent experiments in triplicate. Then, one-way analysis of variance (ANOVA) and multiple comparisons using the Tukey-Kramer test, after assessing data normality with the Shapiro-Wilk test and homoscedasticity with Bartlett's test. For all results, a statistically significant difference was considered if $p<0.05$. Moreover, a principal components analysis (PCA) was also conducted. All statistical analyses were carried out using the JMP v. 18 software.

5. Conclusions

Treatment with ABJ in the in vitro model of colorectal adenocarcinoma lines SW480 and its metastatic derivative SW620 significantly decreases cell viability and proliferative activity, with a greater effect on SW620 cells. Andean berry juice has the potential ability to induce apoptosis in SW480 and SW620 cells through the activation of apoptotic markers, such as the display of phosphatidylserine in the cell membrane and the activity of proteins associated with the extrinsic (TRAIL-DR4) and intrinsic (cytochrome C) pathways of apoptosis, without depolarization of the mitochondrial membrane. The observed biological properties are linked to the complex mixture of polyphenolic compounds, among other components, from the Andean berry, suggesting cytotoxic and pro-apoptotic potential.

 doi:10.20944/preprints202603.2399.v1

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1, Table S2, Table S3, and Figure S1.

Author Contributions: ; I. L. -O.: Software, validation, investigation, data curation, writing-original draft, writing-review and editing, visualization, supervision; M. A. -Q.: Methodology, validation, formal analysis, investigation, data curation, writing-original draft; S. S. A. -V.: Conceptualization, methodology, validation, resources, writing-review and editing; visualization, supervision, project administration, and funding acquisition; S. A. Q.: Validation, resources, investigation, writing-review and editing, funding acquisition; M. E. M. -C.: validation, resources, writing-review and editing; J. A. L. -R.: Conceptualization, software, validation, supervision, writing-review and editing. All authors reviewed and approved the final version of the manuscript.

Funding: This research has been funded by Dirección General de Investigaciones of Universidad Santiago de Cali under call No. DGI-01-2026. This research was funded by Instituto Tecnológico Metropolitano (ITM), grant number P20247; the Committee for the Development of Research-CODI-through Sustainability Strategy of the Research groups 2020-2021 of Universidad de Antioquia, grant number BUPP ES84190022; the Challenge-Based Research Funding (CBRF) Program 2024 from Tecnológico de Monterrey (Grant: CI_EIC_HLT_D_182).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Data Availability Statement: Data will be available upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ΔΨm	Mitochondrial membrane potential.
ABJ	Andean Berry (*Vaccinium meridionale* Swartz) juice.
ACE	Absolute cloning efficiency.
ATCC	American Type Culture collection.
Bad	Bcl-2-associated death promoter.
Bax	Bcl-2 associated X protein.
BCA	Bicinchoninic acid.
Bcl-2	B-cell lymphoma 2.
Bcl-XL	B-cell lymphoma-extra-large.
cIAP-1	Cellular inhibitor of apoptosis protein 1.
cIAP-2	Cellular inhibitor or apoptosis protein 2.
CDKN1A	Cyclin-dependent kinase inhibitor 1A.
CRC	Colorectal cancer.
Cyt. C	Cytochrome C.
DiOC6	3,3-dihexylocarbocyanine iodide.
DMEM	Dulbecco's Modified Eagle's Medium.
DPPH	2,2-diphenyl-1-picrylhydrazyl.
DR4	Death receptor 4.
DR5	Death receptor 5.
FADD	Fas-associated death domain protein.
Fas (CD95)	FS-7 associated protein.
FRAP	Ferric reducing antioxidant power.
FBS	Fetal bovine serum.
FDR	False discovery rate.
HIF1a	Hypoxia-inducible factor 1, alpha subunit.
HMOX2	Heme oxygenase 2.
HPLC-DAD	High-performance liquid chromatography coupled to diode-array detection.

 doi:10.20944/preprints202603.2399.v1

HSP27	Heat-shock protein 27.
HSP32	Heat-shock protein 32.
HSP60	Heat-shock protein 60.
HSP70	Heat-shock protein 70.
HTRA	High-temperature requirement A.
IC_{50}	Half-inhibitory concentration.
KEGG	Kyoto Encyclopedia of Genes and Genomes.
KRAS	Kirsten rat sarcoma viral oncogene homolog protein.
NF-κB	Nuclear factor kappa B.
PI	Propidium iodide.
PCA	Principal components analysis.
PON2	Paraoxonase 2.
RCE	Relative cloning efficiency.
ROS	Reactive oxygen species.
SMAC	Second mitochondria-derived activator of caspase.
SRB	Sulforhodamine B.
SSC	Side scatter.
SW480	Human early colon cancer cells.
SW620	Human metastatic colon cancer cells.
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand.
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A.
SMAC	Second mitochondria-derived activator of caspase
XIAP	X-linked inhibitor of apoptosis protein.

References

1. Tufail, M.; Hu, J.-J.; Liang, J.; He, C.-Y.; Wan, W.-D.; Huang, Y.-Q.; Jiang, C.-H.; Wu, H.; Li, N. Hallmarks of Cancer Resistance. *iScience* **2024**, *27*, 109979, doi:10.1016/j.isci.2024.109979.
2. Sung, H.; Siegel, R.L.; Laversanne, M.; Jiang, C.; Morgan, E.; Zahwe, M.; Cao, Y.; Bray, F.; Jemal, A. Colorectal Cancer Incidence Trends in Younger versus Older Adults: An Analysis of Population-Based Cancer Registry Data. *The Lancet Oncology* **2025**, *26*, 51–63, doi:10.1016/S1470-2045(24)00600-4.
3. Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global Cancer Statistics 2022: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA A Cancer J Clinicians* **2024**, *74*, 229–263, doi:10.3322/caac.21834.
4. Arnold, M.; Sierra, M.S.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Patterns and Trends in Colorectal Cancer Incidence and Mortality. *Gut* **2017**, *66*, 683–691, doi:10.1136/gutjnl-2015-310912.
5. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer Incidence and Mortality Worldwide : Sources , Methods and Major Patterns in GLOBOCAN 2012. **2015**, *386*, doi:10.1002/ijc.29210.
6. Nair, H.H.; Alex, V.V.; Anto, R.J. Significance of Nutraceuticals in Cancer Therapy. In *Evolutionary diversity as a source for anticancer molecules*; Elsevier, 2021; pp. 309–321 ISBN 978-0-12-821710-8.
7. Adebayo, A.S.; Agbaje, K.; Adesina, S.K.; Olajubutu, O. Colorectal Cancer: Disease Process, Current Treatment Options, and Future Perspectives. *Pharmaceutics* **2023**, *15*, 2620, doi:10.3390/pharmaceutics15112620.
8. Kedhari-Sundaram, M.; Raina, R.; Afroze, N.; Bajbouj, K.; Hamad, M.; Haque, S.; Hussain, A. Quercetin Modulates Signaling Pathways and Induces Apoptosis in Cervical Cancer Cells. *Bioscience Reports* **2019**, *39*, BSR20190720, doi:10.1042/BSR20190720.
9. Delgado-Gonzalez, P.; Garza-Treviño, E.N.; De La Garza Kalife, D.A.; Quiroz Reyes, A.; Hernández-Tobías, E.A. Bioactive Compounds of Dietary Origin and Their Influence on Colorectal Cancer as Chemoprevention. *Life* **2023**, *13*, 1977, doi:10.3390/life13101977.
10. Deng, X.; Yang, Z.; Han, M.; Ismail, N.; Esa, N.M.; Razis, A.F.A.; Bakar, M.Z.A.; Chan, K.W. Comprehensive Insights into the Combinatorial Uses of Selected Phytochemicals in Colorectal Cancer Prevention and

 doi:10.20944/preprints202603.2399.v1

Treatment: Isothiocyanates, Quinones, Carotenoids, and Alkaloids. *Phytotherapy Research* **2025**, *39*, 413–452, doi:10.1002/ptr.8378.

11. Ramos-Polo, A.R.; Luzardo-Ocampo, I.; Navarro-Gallón, S.; Quijano, S.A.; Arango-Varela, S.S. Polyphenolic Compounds from Andean Berry (*Vaccinium Meridionale* Swartz) and Derived Functional Benefits: A Systematic and Updated Review. *Foods* **2025**, *14*, 3861, doi:10.3390/foods14223861.
12. Garzón, G.A. Colombian Bilberry (*Vaccinium Meridionale* Swartz): Chemical Composition, Antioxidant Activity , Anthocyanin and Non-Anthocyanin Phenolic Composition as Compared to Other *Vaccinium* Species. In *Berries: Properties, Consumption and Nutrition*; Tuberoso, C., Ed.; Nova Science Publishers, Inc.: New York, 2012; pp. 157–167 ISBN 978-1-61470-257-3.
13. Hernández-Pérez, María Isabel; Lobo-Arias, Mario; Medina-Cano, Clara Inés; Cartagena-Valenzuela, José Régulo *Revista de la Facultad Nacional de Agronomía Medellín*. 2012, pp. 6627–6635.
14. Agudelo, C.D.; Luzardo-Ocampo, I.; Hernández-Arriaga, A.M.; Rendón, J.C.; Campos-Vega, R.; Maldonado-Celis, M.E. Fermented Non-Digestible Fraction of Andean Berry (*Vaccinium Meridionale* Swartz) Juice Induces Apoptosis in Colon Adenocarcinoma Cells. *Preventive Nutrition and Food Science* **2020**, *25*, 272–279, doi:10.3746/pnf.2020.25.3.272.
15. Agudelo, C.D.; Luzardo-Ocampo, I.; Campos-Vega, R.; Loarca-Piña, G.; Maldonado-Celis, M.E. Bioaccessibility during *in Vitro* Digestion and Antiproliferative Effect of Bioactive Compounds from Andean Berry (*Vaccinium Meridionale* Swartz) Juice. *Journal of Agricultural and Food Chemistry* **2018**, *66*, 7358–7366, doi:10.1021/acs.jafc.8b01604.
16. Arango-Varela, S.S.; Torres-Camargo, D.; Reyes-Dieck, C.; Zapata-Londoño, M.B.; Maldonado-Celis, M.E. Aqueous Extract of Andean Berry Induces Apoptosis in Human Colon Cancer Cells without Mitochondrial Damage. *Journal of Berry Research* **2021**, *11*, 377–393, doi:10.3233/JBR-200684.
17. Agudelo, C.D.; Arango, S.; Cortés-mancera, F.; Rojano, B.; Maldonado-Celis, M.E.; Maldonado, M.E.; Carlos, D.A.; Sandra, A.; Fabián, C.-M.; Benjamín, R.; et al. Antiproliferative and Pro-Apoptotic Effects of Andean Berry Juice (*Vaccinium Meridionale*Swartz) on Human Colon Adenocarcinoma SW480 Cells. *Journal of Medicinal Plants Research* **2017**, *11*, 393–402, doi:10.5897/JMPR2017.6401.
18. Arango-Varela, S.S.; Luzardo-Ocampo, I.; Reyes-Dieck, C.; Yahia, E.M.; Maldonado-Celis, M.E. Antiproliferative Potential of Andean Berry (*Vaccinium Meridionale* Swartz) Juice in Combination with Aspirin in Human SW480 Colon Adenocarcinoma Cells. *Journal of Food Biochemistry* **2021**, *45*, doi:10.1111/jfbc.13760.
19. Maldonado-Celis, M.E.; Arango-Varela, S.S.; Rojano, A.B.; Rojano, B.A.; Rojano, A.B. Free Radical Scavenging Capacity and Cytotoxic and Antiproliferative Effects of *Vaccinium Meridionale* Sw. against Colon Cancer Cell Lines. *Revista Cubana de Plantas Medicinales* **2014**, *19*, 172–184.
20. Zapata Vahos, I.C.; Ochoa Agudelo, S.; Alzate Arbelaez, A.F.; Zapata Zapata, A.D.; Rojano, B.A. Production of Vinegar from an Alcoholic Beverage of Andean Berry (*Vaccinium Meridionale* SW), Measurement of 3 Antioxidant Activity and Evaluation of Cytotoxic Effect on 4 Colon Cancer Cells SW480. *Vitae* **2020**, *26*, doi:10.17533/udea.vitae.v26n3a02.
21. Zapata-Vahos, I.C.; Villacorta, V.; Maldonado-Celis, M.E.; Castro-Restrepo, D.; Rojano, B. Antioxidant and Cytotoxic Activity of Black and Green Tea from *Vaccinium Meridionale* Swartz Leaves. *J. Med. Plants Res.* **2015**, *9*, 445–453, doi:10.5897/JMPR2014.5744.
22. Arango-Varela, S.S.; Luzardo-Ocampo, I.; Maldonado-Celis, M.E. Andean Berry (*Vaccinium Meridionale* Swartz) Juice, in Combination with Aspirin, Displayed Antiproliferative and pro-Apoptotic Mechanisms *in Vitro* While Exhibiting Protective Effects against AOM-Induced Colorectal Cancer *in Vivo*. *Food Research International* **2022**, *157*, 111244, doi:10.1016/j.foodres.2022.111244.
23. González, Margarita; Samudio, Ismael; Sequeda-Castañeda, Luis Gonzalo; Celis, Crispín; Iglesias, José; Morales, Ludis Cytotoxic and Antioxidant Capacity of Extracts from *Vaccinium Meridionale* Swartz (Ericaceae) in Transformed Leukemic Cell Lines. *J App Pharma Sci* **2017**, *7*, 24–30, doi:10.7324/JAPS.2017.70305.
24. Sequeda-Castañeda, L.; Barrera-Bugallo, A.; Celis, C.; Iglesias, J.; Morales, L. Evaluation of Antioxidant and Cytotoxic Activity of Extracts from Fruits in Fibroblastoma HT1080 Cell Lines: Four Fruits with Commercial Potential in Colombia. *Emir. J. Food Agric* **2016**, *28*, 143, doi:10.9755/ejfa.2015-11-1007.

 doi:10.20944/preprints202603.2399.v1

25. Andrés-Sánchez, N.; Fisher, D.; Krasinska, L. Physiological Functions and Roles in Cancer of the Proliferation Marker Ki-67. *Journal of Cell Science* **2022**, *135*, jcs258932, doi:10.1242/jcs.258932.
26. Yan, L.; Wei, X.; Zhong, F.; Fu, L.; Ru, H.; Mo, X.; Huang, M. Intratumoral Microbial Community Profiling Identifies Clinicomolecular and Prognostic Subtypes of Colorectal Cancer Liver Metastasis. *npj Precis. Onc.* **2025**, *9*, 284, doi:10.1038/s41698-025-01075-5.
27. Tan, L.; Qu, W.; Wu, D.; Liu, M.; Ai, Q.; Hu, H.; Wang, Q.; Chen, W.; Zhou, H. The Interferon Regulatory Factor 6 Promotes Cisplatin Sensitivity in Colorectal Cancer. *Bioengineered* **2022**, *13*, 10504–10517, doi:10.1080/21655979.2022.2062103.
28. Nissim, N.; Dudaie, M.; Barnea, I.; Shaked, N.T. Real-Time Stain-Free Classification of Cancer Cells and Blood Cells Using Interferometric Phase Microscopy and Machine Learning. *Cytometry Pt A* **2021**, *99*, 511–523, doi:10.1002/cyto.a.24227.
29. Amri, N.; Alaghaz, A.M.A. Synthesis, Structural Investigations, in Vitro Cytotoxicity, Apoptotic Activity, Cell Cycle Analysis, and Molecular Modeling Studies of Nano-sized Zn(II) Schiff Base Complex. *Applied Organom Chemis* **2024**, *38*, e7577, doi:10.1002/aoc.7577.
30. Oshi, M.; Takahashi, H.; Tokumaru, Y.; Yan, L.; Rashid, O.M.; Matsuyama, R.; Endo, I.; Takabe, K. G2M Cell Cycle Pathway Score as a Prognostic Biomarker of Metastasis in Estrogen Receptor (ER)-Positive Breast Cancer. *IJMS* **2020**, *21*, 2921, doi:10.3390/ijms21082921.
31. Verhagen, M.P.; Xu, T.; Stabile, R.; Joosten, R.; Tucci, F.A.; Van Royen, M.; Trerotola, M.; Alberti, S.; Sacchetti, A.; Fodde, R. The SW480 Cell Line as a Model of Resident and Migrating Colon Cancer Stem Cells. *iScience* **2024**, *27*, 110658, doi:10.1016/j.isci.2024.110658.
32. Bause, A.S.; Haigis, M.C. SIRT3 Regulation of Mitochondrial Oxidative Stress. *Experimental Gerontology* **2013**, *48*, 634–639, doi:10.1016/j.exger.2012.08.007.
33. Minker, C.; Duban, L.; Karas, D.; Järvinen, P.; Lobstein, A.; Muller, C.D. Impact of Procyanidins from Different Berries on Caspase 8 Activation in Colon Cancer. *Oxidative Medicine and Cellular Longevity* **2015**, *2015*, 1–13, doi:10.1155/2015/154164.
34. Sri Durgambica, M.; Parimala, K.; Sri Krishna Jayadev, M.; Shanmukha Anand, P.; Srinivasan, T. An Insight into the Therapeutic Potential of Phytochemicals for Colorectal Cancer: Latest Perspective. In *Colon Cancer Diagnosis and Therapy*; Vishvakarma, N.K., Nagaraju, G.P., Shukla, D., Eds.; Springer International Publishing: Cham, 2021; pp. 245–268 ISBN 978-3-030-64667-7.
35. Lee, J.; Kim, Y.-S.; Lee, J.; Heo, S.; Lee, K.; Choi, S.-W.; Kim, Y. Walnut Phenolic Extract and Its Bioactive Compounds Suppress Colon Cancer Cell Growth by Regulating Colon Cancer Stemness. *Nutrients* **2016**, *8*, 439, doi:10.3390/nu8070439.
36. Subramanian, A.P.; John, A.A.; Vellayappan, M.V.; Balaji, A.; Jaganathan, S.K.; Supriyanto, E.; Yusof, M. Gallic Acid: Prospects and Molecular Mechanisms of Its Anticancer Activity. *RSC Advances* **2015**, *5*, 35608–35621, doi:10.1039/c5ra02727f.
37. Vélez-Vargas, L.C.; Santa-González, G.A.; Uribe, D.; Henao-Castañeda, I.C.; Pedroza-Díaz, J. In Vitro and In Silico Study on the Impact of Chlorogenic Acid in Colorectal Cancer Cells: Proliferation, Apoptosis, and Interaction with β-Catenin and LRP6. *Pharmaceuticals* **2023**, *16*, 276, doi:10.3390/ph16020276.
38. Xiao, B.; Deng, X.; Zhou, W.; Tan, E.-K. Flow Cytometry-Based Assessment of Mitophagy Using MitoTracker. *Front. Cell. Neurosci.* **2016**, *10*, doi:10.3389/fncel.2016.00076.
39. Garzón, G.A.; Soto, C.Y.; López-R, M.; Riedl, K.M.; Browmiller, C.R.; Howard, L. Phenolic Profile, in Vitro Antimicrobial Activity and Antioxidant Capacity of Vaccinium Meridionale Swartz Pomace. *Heliyon* **2020**, *6*, e03845, doi:10.1016/j.heliyon.2020.e03845.
40. Garzón, G.A.; Narváez, C.E.; Riedl, K.M.; Schwartz, S.J. Chemical Composition, Anthocyanins, Non-Anthocyanin Phenolics and Antioxidant Activity of Wild Bilberry (*Vaccinium Meridionale* Swartz) from Colombia. *Food Chemistry* **2010**, *122*, 980–986, doi:10.1016/j.foodchem.2010.03.017.
41. Thapa, B.; Kc, R.; Uludağ, H. TRAIL Therapy and Prospective Developments for Cancer Treatment. *Journal of Controlled Release* **2020**, *326*, 335–349, doi:10.1016/j.jconrel.2020.07.013.
42. Vargas-Madriz, Á.F.; Luzardo-Ocampo, I.; Moreno-Celis, U.; Roldán-Padrón, O.; Chávez-Servín, J.L.; Vergara-Castañeda, H.A.; Martínez-Pacheco, M.; Mejía, C.; García-Gasca, T.; Kuri-García, A. Comparison of Phytochemical Composition and Untargeted Metabolomic Analysis of an Extract from Cnidoscolus

 doi:10.20944/preprints202603.2399.v1

Aconitifolius (Mill.) I. I. Johnst and Porophyllum Ruderale (Jacq.) Cass. and Biological Cytotoxic and Antiproliferative Activity in Vitro. *Plants* **2023**, *12*, 1987, doi:10.3390/plants12101987.

43. Kowalczyk, T.; Merecz-Sadowska, A.; Gładys, A.; Osicka, W.; Dudzic, M.; Picot, L.; Piekarski, J.; Szemraj, J.; Rijo, P.; Sitarek, P. The Chemopreventive and Antitumor Potential of Cranberry (*Vaccinium Macrocarpon* Aiton) in Gastrointestinal Cancers: A Review of Molecular Mechanisms, Preclinical Evidence, and Commercial Perspectives. *Food Reviews International* **2025**, 1–29, doi:10.1080/87559129.2025.2597422.
44. Hernández-Zazueta, M.S.; Luzardo-Ocampo, I.; García-Romo, J.S.; Noguera-Artiaga, L.; Carbonell-Barrachina, Á.A.; Taboada-Antelo, P.; Campos-Vega, R.; Rosas-Burgos, E.C.; Burboa-Zazueta, M.G.; Ezquerra-Brauer, J.M.; et al. Bioactive Compounds from Octopus Vulgaris Ink Extracts Exerted Anti-Proliferative and Anti-Inflammatory Effects *in Vitro*. *Food and Chemical Toxicology* **2021**, *151*, 112119, doi:10.1016/j.fct.2021.112119.