

Free radical scavenging capacity and cytotoxic and antiproliferative effects of *Vaccinium meridionale* Sw. against colon cancer cell lines

Capacidad atrapadora de radicales libres, efectos citotóxicos y antiproliferativos de *Vaccinium meridionale* Sw. en líneas celulares de cáncer de colon

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ABSTRACT

Introduction: *Vaccinium meridionale* Swartz, of the family Ericaceae, is commonly known as *mortiño* or *agraz*. The plant is considered a functional food, with a content of anthocyanins and antioxidants similar to or greater than that reported for other *Vaccinium* species. However, little is known about its nutraceutical and medicinal properties.

Objectives: determine the antioxidant activity and cytotoxic and antiproliferative effect of mortiño fruit aqueous extract against colon adenocarcinoma cells (SW480) and their derived metastatic cells (SW620).

Methods: total phenols and anthocyanins were determined by the Folin-Ciocalteu method. Caffeoyl derivatives were determined by HPLC-DAD. Antioxidant activity was analyzed as the ability to scavenge reactive oxygen species (ROS), reactive nitrogen species (RNI), peroxy radicals and hydroxyl radicals. Cytotoxic and antiproliferative activities were studied by MTT and sulforhodamine B.

Results: the following substances were found in 100 g of lyophilized extract: total phenols (2 546 mg GAE), anthocyanins (150.7 mg C3G), chlorogenic acid (126 mg), ferulic acid (108 mg) and coumaric acid (63 mg). Hydroxyl radical scavenging capacity was 36 147.5 μmol DMSO, whereas ROS and RNS scavenging capacity was

29 255.9 y 41 775.2 $\mu\text{molTrolox}$, respectively. ORAC value was 41 775.2 $\mu\text{molTrolox}$. A dose-dependent cytotoxic and antiproliferative effect was observed. IC50 value was 59.12 $\mu\text{g/ml}$ for SW480 and 56.10 $\mu\text{g/mL}$ for SW620.

Conclusions: mortiño fruit aqueous extract exhibited antioxidant, cytotoxic and antiproliferative activities comparable to those of other berries of the genus *Vaccinium*, which could be partly explained by the presence of a high content of anthocyanins and phenolic acids.

Key words: cytotoxic, antiproliferative, free radicals, *Vaccinium meridionale*, colon cancer.

RESUMEN

Introducción: *Vaccinium meridionale* Swartz, pertenece a la familia Ericaceae, conocida comúnmente como mortiño agraz. Considerado un alimento funcional por su contenido de antocianinas y antioxidantes similares o mayor al reportado para otras especies de *Vaccinium*. Sin embargo, poco se conoce sobre sus propiedades nutraceuticas y en la salud.

Objetivos: determinar en extracto acuoso del fruto mortiño, la actividad antioxidante y su efecto citotóxico y antiproliferativo en células de adenocarcinoma de colon (SW480) y sus derivadas metastásicas (SW620).

Métodos: contenido de fenoles y antocianinas totales se determinó por el método Folin-Ciocalteu, los derivados cafeeoil por HPLC-DAD. La actividad antioxidante se analizó como habilidad para atrapar especies reactivas del oxígeno (ROS) y del nitrógeno (RNI), radicales peróxilo e hidróxilo. La actividad citotóxica y antiproliferativa se estudiaron por MTT y sulforodamina B.

Resultados : en 100 g de liofilizado se encontró fenoles totales (2546 mg GAE), antocianinas (150,7 mg C3G), ácido clorogénico (126 mg), ácido ferúlico (108 mg) y cumárico (63 mg), capacidad atrapadora de radical hidroxilo fue 36147,5 $\mu\text{mol DMSO}$, capacidad atrapadora de ROS y RNS fue 29255,9 y 41775,2 $\mu\text{mol Trolox}$, respectivamente, valor ORAC 41775,2 $\mu\text{mol Trolox}$. Se observó un efecto citotóxico y antiproliferativo dosis-dependiente. El valor IC50 para SW480: 59,12 $\mu\text{g/mL}$ y SW620: 56,10 $\mu\text{g/mL}$.

Conclusiones: el extracto acuoso del fruto del mortiño exhibió actividades antioxidantes, citotóxicas y antiproliferativas comparables a las de otras bayas del género *Vaccinium*, lo que podría ser explicado parcialmente por la presencia del alto contenido de antocianinas y ácidos fenólicos.

Palabras clave: citotóxico, antiproliferativo, radicales libres, *Vaccinium meridionale*, cáncer de colon.

INTRODUCTION

Colorectal cancer is the third most common type of cancer and a major cause of death. In the year 2008, approximately 663.904 new cases were diagnosed and 320.397 people died of CRC.¹ The developed countries accounts almost 65 % of the total global incidence, whereas in countries from Central and South America, Asia

and Africa, considered areas of low risk, the incidence is progressively increasing. Several epidemiological studies have shown a consistent association between the low fruit and vegetable consumption and colorectal cancer incidence, which has led to propose the hypothesis that phytochemicals present in these foods may decrease development risk.² This has promoted a greater interest in identifying components of fruits and vegetables with anticancer properties mediated by antioxidant and antiproliferative effects.³⁻⁵

Reactive oxygen species (ROS) are products of the metabolic process. Under normal physiological conditions, the redox state is controlled by the antioxidant system. However, the increase in ROS production, leads to oxidative stress, causing damage to lipids, proteins and nucleic acids, which can lead to different chronic diseases. The high content of polyphenolic compounds in fruits gives them a higher antioxidant potential pharmacological and impart various properties associated with diseases caused by reactive oxygen species (ROS's) inducing leukemia and colon cancers, among others. For example, the anthocyanins in various berries can trap free radicals that cause oxidative stress, and reduce chronic diseases such as various cancers.⁶

The species of the genus *Vaccinium* are a source of polyphenols especially anthocyanins and flavonoids. This fruits contain significant amounts of antioxidant and antiproliferative phytochemicals especially *V. uliginosum*, *V. angustifolium* and *V. myrtillus*, *V. macrocarpon* Ait. The first report about anticancer activity of extracts from cranberrie appeared in 1996 where inhibition of polyamine synthesis and induction of expression of the enzyme quinine reductase was shown.⁷ Subsequently, it was shown that an extract hydrosoluble phenols from a commercial freeze-dried cranberry inhibited the growth of various cancer cell lines of colon: HT-29, HCT-116, SW480 and SW620.⁸ Recently, an extract from the berry *Vaccinium uliginosum* inhibited proliferation of colon cancer cells COLO205 (IC₅₀=50mg /ml).⁹

The above findings have led special interest in the possible role for prevention of various cancers, including colorectal cancer.

Epidemiological studies suggest that consumption of anthocyanins decrease the risk of cancer, partly due to their antioxidant and antiproliferative activities observed *in vitro* assays using colon cancer cell lines.⁸⁻¹² Also in the animal model of colon cancer induced by a zoxymethane or in the family model of adenomatous polyposis (APC^{Min}), extracts rich in anthocyanins from cherry, grape, blueberry, Aronia reduced between 45 to 89 % the number of foci aberrant crypt and adenomatous.¹³⁻¹⁵

Vaccinium meridionale Sw. is a native Colombian plant who belongs to the family of Ericaceae. The fruit is commonly known as mortiño or agraz, is a dark purple globose berry when is ripe. This fruit has a high potential for domestic consumption and has been included in the list of species without wardmarket, called "potential newberry", "Andean blueberry" or "Colombian blueberry". There is a growing interest in this fruit that has been considered a food functional for their content of anthocyanins and antioxidants. Garzón et al. (2010) evaluated the chemical composition, anthocyanin, non-anthocyanin phenolics and phenolic composition of mortiño. Cyanidin 3-galactoside was the major anthocyanin while the most abundant non-anthocyanin phenolic was chlorogenic acid.¹⁶ Gaviria et al. (2009) evaluated the content of phenols and anthocyanins and antioxidant activity by different methodologies and found similar or higher values than those reported for other species of *Vaccinium*.¹⁷ Moreover, non-ethanolic extracts of *Vaccinium meridionale* Swartz rich in anthocyanins showed cardioprotective activity in rats during an ischemia-reperfusion process mediated by reactive oxygen species.¹⁸

However, due to few information on its potential as a nutraceutical food and health applications, in this study we analyzed for the first time in the aqueous extract of mortiño the antioxidant activity by fluorescence methods expressed as the ability to trap total reactive oxygen species (ROS) and reactive nitrogen species RNS, peroxy, hydroxyl radicals and their effects on the viability and growth of primary tumor cells of colon cancer (SW480) and their metastatic-derived cells (SW620), considered an *in vitro* model representing colon cancer progression to metastatic disease.¹⁹

METHODS

Chemicals. Milli-Q- water (Millipore, Bedford, MA) was used in all work, HPLC - grade acetonitrile, phosphoric and formic acid (Merck, Darmstadt, Germany) were used after filtration through a 0.45 µm pore size membrane filter, 2,20-Azo-bis (2-amidinopropane) dihydrochloride (AAPH), fluorescein, 2,7-diclorofluoresceína diacetate, sodium terephthalate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol and other HPLC solvents were from were purchased from Merck (Darmstadt, Germany). Cyanidin 3-glucoside and cyanidin 3-arabinoside were purchased from Indofine Chemical Co., Inc. (Somerville, NJ, USA).

Plant sample collection: healthy mortiño or agraz berries were harvested from Germoplasma *Vaccinium* collection in the Center of Research "La Selva" of Corpoica, municipality of Rionegro (Antioquia) at 2 120 msnm, 17 °C and 78 % relative humidity. This place belongs to the living area of lower montane rain forest and is located at 06° 08' 06'' north latitude and 75° 25' 03'' west longitude. This material has a voucher number ILS 14050070. Fruit samples were transported to laboratory in plastic bags seal and washed in distilled water.

Preparation of aqueous extract: to obtain the aqueous extracts, 20 g of edible portions from healthy fruits were mixed with 50 mL of distilled water quality, homogenized in a Ultra Turrax® (Ika-Werke, Staufen, Germany) at 15000 rpm for 20 sec, filtered using a No.1 Whatman paper filter and then freeze-dried in a Labconco, Freezome 2-5 Plus System (Fisher Scientific, Pittsburg). The freeze-dried materia from fruits, equivalent to instant juice were kept at -20 °C in plastic tubes, sealed with parafilm and protected from light. Immediately before use, the extracts were sterilized through a 0.22 µm syringe filter.

Determination of total phenols and total anthocyanins: total phenolic content was measured by using the Folin-Ciocalteu. Results were expressed as gallic acid equivalents/mg of lyophilized. Total anthocyanins were determined by using a pH differential method. Results were expressed as mg of cyanidin-3-glucoside equivalents/mg of lyophilized.²⁰

Caffeoyl derivatives determination by HPLC-DAD: Hydroxycinnamic acids were analyzed by direct injection of the samples, previously filtered through a 0.45 µm pore-size nylon filter, in a HPLC-DAD using a Shimadzu LC-20AD/T HPLC equipped with a SPD-6AUV detector (Kyoto, Japan) and a Pinnacle (II) C18 column (5 µm) 250 x 4.6 mm (Restek®, Bellefonte, USA) with an autoinjector and a photodiode array detector (PDA). Chlorogenic, caffeic, ferulic and p-coumaric acid were adopted as the standard for identification and quantification of hydroxycinnamic acids at 320 nm. The mobile phase was a sample of 10 µL of a mixture of acetonitrile, acidified water (phosphoric acid at pH = 2.5) (40:60) v/v, supplied at a rate of 0.8 mL/min.²¹

ORAC assay: the ORAC assay was determined by the following methodology. 3 mL were prepared from the following solution: 21 μ L of a 10 μ M solution of fluorescein, 2899 μ L of 75 mM phosphate buffer (pH 7.4), 50 μ L of 600 mM AAPH and 30 μ L of extract. Fluorescence was recorded on a Perkin Elmer LS45 spectrofluorometer with a thermostated multicell. The ORAC value μ MolTrolox/100g lyophilized was calculated by a calibration curve using different concentrations of Trolox[®].²²

Total capacity assessment to trap reactive oxygen species and (ROS's) : this assay evaluates the antioxidant capacity extract to trap reactive oxygen species (ROS's), which are generated by the azo compound, 2,2'-azobis-2-methylpropanimidamide, dihydrochloride (AAPH), in aqueous medium that produces free radicals at a rate constant. The reaction was carried out in 75 mM phosphate buffer, pH 7.4. 50 μ L of mixed solution of AAPH 0.3 M, 50 μ L of an ethanol solution of 2,7-dichlorofluorescein diacetate 2.4mM, 2850 μ L of buffer and 50 μ L of the test sample. Immediately reads the intensity of fluorescence emitted during the first 10 minutes and compared to the intensity emitted in the absence of the sample. The results are expressed as TEAC values (mg trolox/100 g of extract, by constructing a calibration curve using different concentrations of Trolox[®].²³

Fluorescence probe for detection of hydroxyl radical: the hydroxyl radical scavenging activity was determined by the following methodology. The reaction was carried out in 0.2 M phosphate buffer, pH 7.4. Mix 300 μ L of a solution of sodium terephthalate 1×10^{-4} M, 2420 μ L of buffer, 100 μ L of the test sample, 90 μ L of a solution of 1×10^{-2} M EDTA and finally 90 μ L of a solution of Fe^{+2} 1×10^{-2} M. The mixture was allowed to stand for 6 minutes with constant aeration at room temperature. Results are expressed as μ mol of DMSO/100 g of lyophilized, by constructing a calibration curve using different concentrations of DMSO.²⁴

Cell culture: SW480 and SW620 cells were obtained from the European Collection of Animal Cell Culture (ECACC, Salisbury, UK). They cultured according to a previously described procedure.²⁵ Cell were cultured in 75 cm² Falcon flasks with Dulbecco's modified Eagle's medium supplemented with 25 mM glucose, 2 mM L-glutamine, 10 % heat (56 °C)-inactivated horse serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 1 % non-essential amino acids. Incubations were carried out at 37 °C in a humidified atmosphere with 5 % CO₂. The culture medium was replaced every 48 h. For all experiments, horse serum was reduced to 3 %, and the medium was supplemented with 10 μ g/mL insulin, 5 μ g/mL transferrin and 5 ng/mL selenium (ITS defined medium). Cells were exposed to different extracts 24 h after seeding.

MTT assay: the cytotoxic activity of extracts was screened in colon cancer cells by using the MTT assay.²⁶ This is based on the conversion of yellow tetrazolium salt MTT to purple formazan crystals by metabolically active cells. The amount of formazan produced is proportional to the number of viable cells, a product generated by the activity of dehydrogenases. In brief, 3000 viable cells from each cell line were seeded in a 96-well plate (Falcon), then 24 h after seeding the medium was replaced and the cells were treated with the extracts at different concentrations (0 - 400 mg/mL) dissolved in ultrapure water. After 48 h of incubation 10 μ L of 5 mg/ml MTT solution were added to each well and incubated at 37 °C for 4 h in darkness. The formazan crystals were dissolved by adding 80 μ L of acidified isopropanol (0.4 N HCl) to each well shaking continuously in darkness at room temperature. The amount of MTT-formazan that is directly proportional to the number of living cells was determined by measuring the optical density (OD) at 540 nm using a microplate ELISA reader (GloMax [®]-Multi Promega) and at 750 nm reference wavelength. Negative control was non-treated cells. The concentration of the extracts that killed 50 % of the cells (IC₅₀) was calculated using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The percentage inhibition of

viability compared to the negative control was calculated using the following equation: % Inhibition = $[1 - (OD_t / OD_c)] \times 100$. Where OD_t is the optical density of treated cells and OD_c is the optical density of control cells. All experiments were performed in triplicate.

Sulforhodamine B (SRB) assay: the effect on growth rate of aqueous extract in colon cancer cell lines was described by using the SRB assay, a colorimetric assay that estimates cell number indirectly by staining total cellular protein with SRB dye.²⁷ In brief, 3000 viable cells from each cell line were seeded and treated in a 96-well plate (Falcon) as described for MTT assay. After 48 h incubation, the medium was discarded and cells were fixed by adding 50 μ L ice-cold 50 % v/v trichloroacetic acid (Merck). The cells were then incubated at 4 °C for 1 h and the plates were washed five times with water at room temperature. The excess water was drained off and the plates were left to dry overnight. Then, 200 μ L of SRB stain (0.4 % w/v in 1 % acetic acid) were added to each well for 30 min. Finally, the plates were washed with 50 mL 1 % acetic acid, and rinsed four times until dye adhering to the cells was observed. The relationship between cell number (protein content/well) and absorbance is linear from 0 to 2×10^5 cells per well. The absorbance of SRB is proportional to the number of adherent and live cells were determined by measuring the optical density (OD) at 490 nm using a microplate ELISA reader (GloMax ®-Multi Promega). All experiments were performed in triplicate.

Statistical Analysis : all data were presented as mean \pm standard deviation (SD) or mean \pm standard error (SE) from three independent experiments. Non-linear regression analysis was used to calculate IC_{50} . Statistical differences between cells treated and non-treated (control) groups were evaluated by one-way ANOVA and specific differences were identified using the Tukey test's ($p < 0.05$) using GraphPad Prism version 5 for Windows (Graph Pad Software, San Diego, California, USA).

RESULTS

Composition and scavenger activity of lyophilized agraz.

Composition: Total anthocyanins: 150.7mg of cyanidin-3-glucoside equivalents/100 g of lyophilized; total phenols, 2546 mg of gallic acid equivalents/100 g of lyophilized. Phenolic acids such: chlorogenic: 126 mg/100 g of lyophilized; ferulic: 108 mg/100 g of lyophilized, coumaric: 63/100 g of lyophilized.

Antioxidant activity : the antioxidant activity was measured as: hydroxyl radical scavenging capacity: 36147.5 ± 6274.7 (μ molDMSO/100g lyophilized), total scavenger capacity to ROS and RNS: 29255.9 ± 6531.27 μ mol Trolox / 100 g lyophilized), 41775.2 ± 6168.2 μ mol Trolox/100 g lyophilized) and ORAC value: $41775.2 \pm 6168,2$. μ molTrolox/100 g lyophilized).

Cell viability: the cytotoxic effect of *V. meridionale* berry extract against SW480 and SW620 cells was determined by MTT assay and results of response to different concentrations (25 - 400 μ g/mL) are shown in table where the inhibitory effect on cell viability on SW480 and SW620 increased significantly ($p < 0.05$) as concentration increased from 25 to 400 μ g/mL. The IC_{50} value calculated from the non-linear regression between percent of inhibition and logarithm concentration was found SW480: $IC_{50} = 59.12$ μ g/mL and SW620: $IC_{50} = 56.10$ μ g/mL.

Table 1. Percent of inhibition of *Vaccinium meridionale* Swartz berry aqueous extract on cell SW480 and SW620 viability. The result not sharing the same superscript letter differ significantly: a≠b≠c≠d≠e and a'≠b'≠c'≠d', p<0.05

Colon Cancer cell lines	Inhibition of cell viability (%)					
	Extract concentration (µg/mL)					
	25	50	100	200	400	IC ₅₀
SW480	13,90±0.78 ^a	27,33±0.39 ^b	52,60±0.47 ^c	67,50±0.23 ^d	76,67±1.63 ^e	59.12
SW620	15,7±1.36 ^{a'}	40,8±3.83 ^{b'}	58,5±1.07 ^{b'}	73,1±0.74 ^{c'}	91,3±1.31 ^{d'}	56.10

The effect of *V. meridionale* extract on SW480 and SW620 cell growth is represented in Figure as OD of cell proteins at 490 nm treated or not with the extract at different concentrations (50 to 200 µg/mL). The OD of SW480 cell protein was reduced between by 27.5, 53.4, and 65.8 % at 50, 100, and 200 µg/mL aqueous extract concentration respectively compared to non-treated SW480 cells (control) after 72 hours of treatment. Related to SW620 cells *V. meridionale* aqueous extract at same concentrations reduced by 20, 41, and 71 % respectively the OD of cell proteins stained with SRB compared to SW620 non-treated cells.

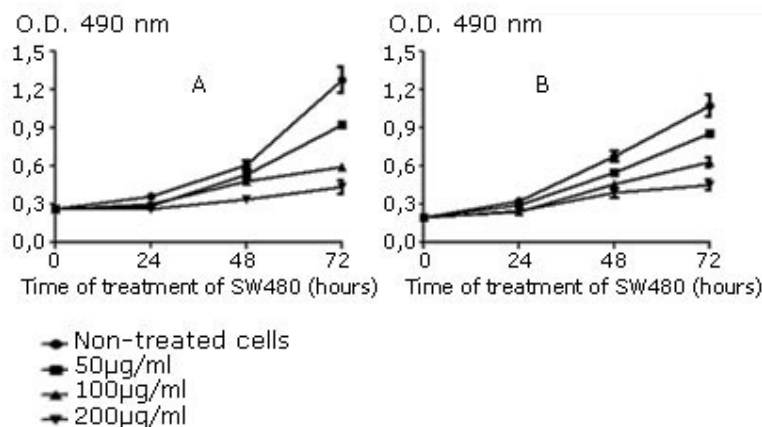


Fig. Effect of aqueous extract of *Vaccinium meridionale* Sw. berry on SW480 (A) and SW620 (B) cell growth.

DISCUSSION

The species of the genus *Vaccinium* are rich in phenolic compounds, especially anthocyanins. *V. meridionale* has a high content of phenolic compounds and anthocyanins, similar or higher than most reported species of berries and other fruits. The results in this work, are comparable to anthocyanins and phenolic compounds content of *V. corymbosum* (Northern Highbush blueberry: 92-235 C3G and 181-473 GAE); *V. ashei* (Rabbiteye blueberry, 60-187 C3G and 230-457 GAE), *V. angustifolium* (Lowbush blueberry, 190-300 C3G and 290-495 GAE).¹⁷ The phenolic structure of anthocyanins is responsible for their antioxidant activity i.e., ability to scavenge reactive species (ROS and RNS) such as superoxide, singlet oxygen, hydrogen peroxide, hydroxyl radical. Total ORAC value found for Mortiño is higher than most grains, vegetables and fruits, including many types of cherries, strawberries, grapes, blueberries, and less than extracts from acai (*Euterpeoleraaceae*) and passion fruit.²⁴

Most of the protective effects of anthocyanins are attributed to their ability to scavenge ROS, they also function by chelating metals and by direct binding to proteins. The antioxidant effects of anthocyanins in vitro have been demonstrated in several cell culture systems of colon. In these culture systems, anthocyanins exhibited antitoxic and anti-carcinogenic effects such as: directly scavenging reactive oxygen species (ROS), increasing the oxygen-radical absorbing capacity of cells, stimulating the expression of enzymes, reducing the formation of oxidative adducts in DNA, decreasing lipid peroxidation, inhibiting mutagenesis by environmental toxins and carcinogens, and reducing cellular proliferation by modulating signal transduction pathways.^{28, 29}

Cytotoxic activity was evaluated in order to know the ability of an aqueous extract of *V. meridionale* berry to affect the viability of human colon adenocarcinoma (primary tumor) SW480 cells and their metastatic-derived cells SW620 isolated from a mesenteric node in the same patient.³⁰ The inhibitory effect of *V. meridionale* berry extract against SW480 and SW620 cells increased significantly ($p < 0.05$) as concentration increased from 25 to 400 $\mu\text{g/ml}$. The IC_{50} value was found to be similar in both colon cancer cell lines.

Antiproliferative activity of aqueous extract obtained from *V. meridionale* berry was evaluated to know if cytotoxic effect observed on SW480 and SW620 cells might be associated induction of cell death or/and suppression of cell proliferation. This analysis was performed by SRB assay after cell treatment with different concentrations of the extract for 72 hours and results were represented in Figure 1 as OD of cell proteins at 490 nm treated or not with the extract at different concentrations (50 to 200 mg/mL. These data indicate that *V. meridionale* aqueous extract was able to reduce the cell growth in a similar way colon adenocarcinoma (SW480) and metastatic cells (SW620) which suggest that *V. meridionale* berry has antiproliferative activity by reducing SW480 and SW620 cell growth.

This study show by first time cytotoxic and antiproliferative properties present in an aqueous extract obtained from the *V. meridionale* berry. Results obtained against two colon cancer cell lines (primary tumor SW480 and metastatic SW620) indicated that this Colombian berry fruit is able to affect cell viability by reducing or suppressing cell growth. These findings are contrary to that observed by Seeram (2004) using a total crude extract of *V. macrocarpon* which inhibited by 35 % cell growth of SW620 but not SW480, whereas HT29 and HCT116 were affected by 78 and 58 % respectively. By other hand, was reported that using enriched extracts in anthocyanins obtained from *V. myrtillus* berry growth cell of HT29 was inhibited by 7 % at 200mg/ml and, by 34 % and 3 % in HCT116 at 4 mg/ml and 2 mg/mL, respectively.^{31,32} Subsequently, it was also found that an enriched fraction of anthocyanins of *V. ashei* Readeberry was more cytotoxic (IC_{50} between 15 - 50 mg/mL) against CaCo-2 and HT29 cells than enriched fractions in tannins (IC_{50} between 50- 100 mg/mL) or phenolic acids ($\text{IC}_{50} = 1000$ mg/mL).^{11,33}

In our study, the cytotoxic effect of aqueous extract of *V. meridionale* berry seems to be associated with the inhibition of SW480 and SW620 cell growth. The aqueous extract showed a cytotoxic effect higher (73 and 78 % respectively) and similar IC_{50} values (59 and 56 mg/mL) compared to those described for these and other colon cancer cell lines using extracts of berries from genus *Vaccinium*. The antiproliferative effect was evidenced by a progressive decrease in cell growth of SW480 and SW620 in time at different concentrations of the aqueous extract (50 - 200 mg/mL) reaching an inhibition of 65.8 and 71 % after 72 hours of treatment at 200 mg/mL, respectively. Similar to the cytotoxic effect, the inhibition of cell growth was higher in SW620 cells than in SW480 cells, a similar behavior has been observed with other plant extracts in these cell lines.^{25,33,34} A possible explanation for the difference in sensitivity of these cell lines could be the changes acquired as

they progress to the metastatic phenotype which becomes a good model for representing the progression of colon carcinogenesis from a primary tumor to metastatic disease, useful for evaluate selectivity property of potential chemopreventive agents.^{19,30,35}

The components of *V. meridionale* berry extract responsible of the anticarcinogenic effects described here are unknown. However considering that our results were similar to those ones described using anthocyanins enriched fractions from different *Vaccinium* berries against colon cancer cell lines; we propose that cytotoxic and antiproliferative effects of *V. meridionale* berry on SW480 and SW620 cells might be attributed to these polyphenols.^{11,31,32, 36} The anthocyanins are flavonoids widely distributed and of great importance for their chemopreventive activity against colorectal cancer observed *in vitro* and *in vivo* studies.²⁹ By other hand, it is possible that the cytotoxic and antiproliferative effects against SW480 and SW620 cells were due to the synergistic action of anthocyanins with other important compounds present in *V. meridionale* berry such as phenolic acids compounds which comprises 30 % of dietary polyphenols.³

Phenolic acids compounds can be subdivided in two major groups, derivatives of hydroxybenzoic acids and derivatives of hydroxycinnamic acids.^{37,38} The last ones have been associated with a protective effect on CRC.³⁹ Yi et al⁴⁰ found that a phenolic acid fraction of rabbiteye blueberries inhibited 50 % proliferation of colon adenocarcinoma cell lines HT-29 and CaCo-2 at 1000 mg/ml for 72h of treatment, indicating a relatively low bioactivity. In other hand, chlorogenic acid, the main hydroxycinnamic acid, has received significant attention as chemopreventive agent. Veeriah et al.⁴¹ and Gleit et al.⁴² found that 500µmol/L and 289.2µmol/L chlorogenic acid from apple and coffee, respectively inhibited 50 % of HT29 cell viability after 72h of treatment, and recently Thurow⁴³ reported that 150µmol/L of chlorogenic acid from prune (*Prunus domestica* L.) reduced CaCo-2 cell growth by 63 % after 24h of treatment. Other hydroxycinnamic acids and derivatives such as caffeic acid phenyl ester, ferulic acid, p-coumaric acid and caffeoylquinic acids have showed anticarcinogenic activities against HT-29, CaCo-2, SW480, HCT116 involving different mechanisms such as cell growth reduction, cycle arrest, and apoptosis.⁴⁴⁻⁴⁷

Respect to the mechanisms involved in the antiproliferative effects of the aqueous extract of *V. meridionale* are unknown, but taking account that the antiproliferative activity of these polyphenols may occur by cell-cycle arrest and apoptosis, as mentioned. To answer this question we are performing additional studies in our laboratory to know if cell-cycle arrest and apoptosis are involved in *V. meridionale* berry-induced cell death of colon tumor primary and metastatic cells.^{29, 3, 4}

In conclusion, the present study shows that an aqueous extract obtained from the Colombian berry *V. meridionale* exhibited antioxidant, cytotoxic and antiproliferative activities as well as described for other berries from genus *Vaccinium*. The antioxidant and anticancer activities could be explained partly by the presence of high content of anthocyanins and phenolic acids by their comparable concentration to other *Vaccinium* berries. The finding that *V. meridionale* aqueous extract inhibits viability and growth on a *in vitro* model of colon cancer progression from adenocarcinoma to metastatic disease may suggest a potential of this fruit for the prevention of CRC *in vivo*.

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