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Abstract

Background: Extracts from a variety of fruit trees have been used for therapeutic applications for preventing oxidative stress associated to chronic diseases. **Objective:** To investigate the antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits of *Passiflora edulis*. **Materials and methods:** A preliminary phytochemical screening was performed; antioxidant activity was evaluated through DPPH assay, the scavenging activity for hydroxyl radical, antihemolytic activity and total phenolic content; cytotoxic and antiproliferative activities were evaluated by MTT and sulforhodamine B assays respectively in colon adenocarcinoma SW480 cells and their metastatic-derived SW620 cells. **Results:** Phytochemical analyses revealed presence of tannins, flavonoids and cardiogenic glycosides. Ethanolic extract from leaves showed the best antioxidant activity ($EC_{50} = 0.096$ mg/ml) in the DPPH assay and the juice ($EC_{50} = 0.022$ mg/ml) for the Hydroxyl free radical-scavenging activity. All extracts inhibited more than 98% the hemolysis induced by H_2O_2 . The aqueous extract from leaves showed the highest cytotoxic activity against SW480 and SW620 cells. **Conclusions:** Findings from this study suggest that *P. edulis* is a potential source of phytochemical compounds with antioxidant and antiproliferative properties.

Key words: *Passiflora edulis*, antioxidant, antiproliferative, cytotoxic.

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Actividad antioxidante y antiproliferativa de extractos etanólico y acuoso de las hojas y el jugo del fruto de *Passiflora edulis*

Resumen

Antecedentes: extractos de varios árboles frutales se han utilizado en diferentes aplicaciones terapéuticas para prevenir el estrés oxidativo asociado a enfermedades crónicas. **Objetivo:** investigar la actividad antioxidante y antiproliferativa de extractos etanólico y acuosos de las hojas y del fruto de *Passiflora edulis*. **Materiales y métodos:** se realizó marcha fitoquímica preliminar, la actividad antioxidante se evaluó por DPPH, actividad de remoción de radicales hidroxilo, actividad antihemolítica y contenido fenólico total. En células de adenocarcinoma de colon SW480 y sus derivadas metastásicas SW620 se evaluó la actividad citotóxica y antiproliferativa por el método de MTT y sulforodamina B respectivamente. **Resultados:** el análisis fitoquímico reveló la presencia de taninos, flavonoides y glicósidos cardiotónicos. El extracto etanólico de las hojas mostró la mayor actividad antioxidante ($EC_{50} = 0,096$ mg/ml) por DPPH y el jugo ($EC_{50} = 0,022$ mg/ml) para remover el radical hidroxilo. Todos los extractos inhibieron más del 98% la hemólisis inducida por H_2O_2 . El extracto acuoso de las hojas mostró la mayor actividad citotóxica y antiproliferativa contra células SW480 y SW620. **Conclusiones:** los hallazgos de este estudio sugieren que *P. edulis* es una fuente potencial de compuestos fitoquímicos con propiedades antioxidante y antiproliferativa.

Palabras clave: *Passiflora edulis*, antioxidante, antiproliferativo, citotóxico.

INTRODUCTION

Oxidative stress has been associated with cancer, among other diseases. This process is caused by an imbalance between the production of reactive oxygen species (ROS) and the availability of biological antioxidants, which leads to cell damage (1).

Phytochemicals constitute an important class of plant-derived compounds with beneficial health properties because of their antioxidant and free-radical scavenging abilities among other biological properties (2). The most abundantly occurring plant-derived antioxidants are polyphenols (3). Although scientific interest in these compounds is relatively recent, these have been used for centuries as a strategic alternative for the prevention and / or combined treatment of various diseases (4).

Antioxidants may protect against cancer through different mechanisms involving up-regulation of antioxidant enzymes, repair of nuclear DNA, and apoptosis (5). *Passiflora edulis* is a Brazilian native plant, known as passion fruit which belongs to Passifloraceas (6). Different parts of this plant have been used in traditional medicine for the treatment of insomnia, sedation, epilepsy, hypnotic, hypertension, diuretic, reduction of cholesterol and triglycerides, bronchitis, asthma, colds, antispasmodic, stomach pain, tetanus, boils, and intestinal tumors (7-10). Currently, plant-derived antioxidants have become an interesting focus of research, with the aim of finding compounds that provide cellular protection against oxidative stress and cancer development. Therefore, this study we investigated the antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits of *Passiflora edulis* (passion fruit).

MATERIALS AND METHODS

Extracts preparation: Healthy *P. edulis* leaves and fruits were collected between 6:00 and 8:00 am on Mirador farm in the village of La Herradura, municipality of La Tebaida, Armenia, Quindío (4.4376° N, 75.8489° W), 1165 msnm. The specimens were identified by the Herbarium of Universidad del Quindío (collection number: 33974). Plant samples were transported to laboratory in sealed plastic bags and washed in distilled water. Leaves were dried at 40°C until they reached a constant weight and pulverized with a mill. The ethanolic extract was obtained using the leaves' powder in 96% ethanol (EtOH). The chlorophyll was separated with EtOH/water (11). The leaves aqueous extract was prepared dissolving the leaves powder in distilled water at 40°C and filtered. The resulting ethanolic and aqueous extracts were evaporated in a Heidolph® rotary evaporator. A stock solution was prepared in 20% solution and stored at 4°C until use. To obtain the juice, fruits pulp and seeds were sieved (pore size 1 mm), the pulp was evaporated to dryness in a Heidolph® rotary evaporator and dissolved in distilled water to obtain a stock solution of 1mg/ml stored at 4°C until use.

Preliminary phytochemical screening: This qualitative test was performed to identify secondary metabolites (tannins, flavonoids, quinones, sterols, saponins, cardiotonic glycosides and carotenoids) (12).

DPPH Radical scavenging activity: This assay was performed according to the method of Brand-Williams et al. (13) modified by Gunjan et al. (14). For each extract at 0.1 mg/ml; 0.2 mg/ml; 0.5 mg/ml and 1 mg/ml a DPPH solution (0.2 mM in methanol) was added (Sigma-Aldrich, United States). The mixture was incubated in darkness at 20°C for 40 min. Absorbance was measured at 517 nm using methanol as a blank. A positive control, butylated

hydroxy toluene (BHT), was used in methanol at the same concentrations of the extracts. Distilled water was used as a negative control. The percentage inhibition level of DPPH by the different extracts was calculated according to the following equation: % Radical scavenging = $[(A_c - A) / A_c] \times 100$ where A_c is the absorbance of the negative control and A is the absorbance of sample (extract or BHT).

Antihemolytic activity: Was evaluated according to the procedure described by Nabavi et al. (15). Briefly, blood samples from healthy volunteer donors for hemolytic diseases were obtained by venipuncture in tubes without anticoagulant. Blood was centrifuged at 2500 rpm for 10 min, plasma and leukocytes were removed. Erythrocytes were washed three times with phosphate buffered saline (PBS) pH 7.4. A 5% (v/v) suspension of erythrocytes in PBS was added each extracts at 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml and 1mg/ml, incubated at 37°C for 3 hours. After centrifuge mixture reactions, supernatant was obtained and diluted in PBS. Absorbance of resulting hemoglobin in supernatants was measured at 540 nm. Erythrocytes in PBS and vitamin C were used as negative and positive controls, respectively (Sigma-Aldrich, United States). The percentage inhibition of hemolysis was calculated using the following equation: % Inhibition of hemolysis = $[(A_c - A) / A_c] \times 100$. Where A_c is the absorbance of negative control and A is the absorbance of the sample (extract or vitamin C).

Total phenolic content: Was performed using the Folin-Ciocalteu method (14). To each extract, Folin-Ciocalteu reagent (Sigma-Aldrich, United States) and 7.5% Na_2CO_3 were added, mixed and incubated at 40°C for 15 minutes. The absorbance of samples was measured at 765 nm using 7.5% Na_2CO_3 as the blank. Data were expressed as mg gallic acid equivalents in gram of extract (mg GAE eq / g extract), according to a calibration curve.

Hydroxyl free radical-scavenging activity

(HRSA): Was measured according to the method of Yang et al. (16). Briefly, to each extract at different concentrations was added 2 mM FeSO₄, 6 mM salicylic acid, and 0.01% H₂O₂, incubated at 37°C for 1 hour. Ascorbic acid was used as a positive control (Sigma-Aldrich, United States). The control containing all reagents but sample was used as a blank. The HRSA was determined by measuring the absorbance at 510 nm. The percentage of HRSA was calculated with the following equation: % HRSA = [(A0 - As) / A0] x 100. Where A0 is the absorbance of negative control and As is the absorbance of samples (extract or ascorbic acid).

Total antioxidant capacity: Was determined according to the method of Prieto et al. (17). For each extract at different concentrations 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added and incubated at 95°C for 90 minutes. The absorbance was measured at 695 nm against a blank. Antioxidant total capacity was expressed as mg equivalents of gram Butyl Hydroxytoluene (mg equiv/g BHT) (Sigma-Aldrich, United States), against a calibration curve.

Cell culture: SW480 and SW620 cells were obtained from the European Collection of Animal Cell Culture (ECACC, Salisbury, UK). They were cultured according to a previously described procedure (18). Briefly, cells were cultured in medium DMEM supplemented with 10% horse serum (HS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 1% non-essential amino acids (Invitrogen, Cergy-Pontoise, France). For all experiments, cells were switched to assay medium containing 3% HS, and 10 µg/ml insulin, 5 µg/ml transferrin, and 5 ng/ml selenium (Invitrogen) for extracts administration 24 h after seeding.

MTT assay: Cytotoxic activity of extracts was performed in SW480 and SW620 (19). This method

is based on the conversion of tetrazolium salt to formazan which is proportional to viable cells, a product generated by the activity of mitochondrial dehydrogenases. In brief, 3000 viable cells from each cell line were seeded in a 96-well cell culture plate after 24 hours, then the medium was replaced with fresh assay medium containing dilutions of extracts (0 - 400 µg/ml) dissolved in DMSO or 0.1% DMSO final concentrations, respectively for 72 h. Then, a MTT solution (5 mg/ml) was added to each well and incubated at 37°C for 4 hours in darkness. The formazan crystals were dissolved by adding acidified isopropanol (0.4 N HCl) to each well shaking continuously in darkness at room temperature (RT). Absorbance was measured at a 540 nm and 750 nm reference wavelength. The concentration able to kill 50% of cells (IC50) was calculated using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The absorbance of control group (non-treated cells) was considered as 100% viability. The percent inhibition was calculated using the following equation: % Inhibition = [1 - (ODt / ODc)] x 100. Where ODt is the optical density (OD) of treated cells, and ODc for control (non-treated cells).

Sulforhodamina B (SRB) assay: The effect of extracts on growth cells was studied by using the SRB assay according to Gossé et al. (20), a colorimetric assay based on staining of total cellular protein from cells with SRB dye. In brief, 3000 viable cells from each cell line were exposed to extracts for 24 hours after seeding and incubated for different times. Control cells were treated with 0.1% DMSO. Culture media were replaced every 48 hours. The cell culture was stopped by the addition of trichloroacetic acid (50% v/v), and cell proteins were determined by staining with 0.4% (w/v) SRB (Sigma-Aldrich, United States). The relationship between cell number (protein content/well) and absorbance is linear from 0 to 2x10⁵ cells per well. All experiments were performed in triplicate.

Statistical analysis: Data were presented as mean \pm standard deviation (SD) of at least three independent experiments. Linear regression analysis was used to calculate effective concentration (EC_{50}) or IC_{50} and inhibition percentage correlation (dose-response). Statistical differences between groups were evaluated using ANOVA with repeated measures and p values were adjusted by Bonferroni correction ($P < 0.05$) using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

RESULTS

Preliminary phytochemical screening: Table 1 shows presence of tannins, flavonoids, quinones, carotenoids, cardiotoxic glycosides and sugars in all extracts. Saponins were absent in all extracts. Sterols just were present in the juice.

Table 1. Phytochemical screening of extracts from *P. edulis*

Phytochemical constituents	Ethanolic	Aqueous	Juice
Tannins	++	+	++
Flavonoids	++	++	+
Quinones	++	+	+
Esterols	-	-	+
Saponins	-	-	-
Cardiotoxic glycosides	++	+++	+++
Carotenoids	+++	+	+++
Carbohydrates	++	+++	+++

(+) little presence, (++) mild presence, (+++) high presence, (-) absent.

DPPH Radical Scavenging Activity: Figure 1 shows the percentage of scavenging is directly proportional to the concentration of each extract (ethanolic: $r^2 = 0.672$ ($p < 0.008$), aqueous: $r^2 = 0.906$ ($p < 0.001$); juice: $r^2 = 0.413$ ($p = 0.051$)).

The ethanolic extract showed the strongest activity (87.4%), followed by the aqueous extract (73.4%) and the juice (15.6%), being statistically significant between them ($p < 0.016$). No statistical difference was observed between the results obtained with the ethanol extract and positive control ($p = 0.243$). The EC_{50} for the ethanolic, aqueous and juice extracts was respectively 0.096 mg/ml, 0.448 mg/ml and 3.5 mg/ml. In addition, the ethanolic extract at 0.5 mg/ml and 1.0 mg/ml showed a DPPH value similar to the positive control (BHT) considered a highly effective antioxidant.

Antihemolytic activity: In figure 2, ethanolic and aqueous extracts shows more ability to inhibit the hemolysis H_2O_2 -induced than positive control. The antihemolytic activity of juice was the lowest compared to the other extracts and significantly different respect to positive control ($p < 0.001$). In addition, the antihemolytic effect using the juice was dose-dependent.

Total phenolic content: Table 2 shows that the ethanolic and aqueous extracts presented the highest concentration of phenolic compounds, 429 and 368 mg GAE/g extract respectively.

Table 2. Total phenolic content and antioxidant capacity of *P. edulis* extracts

Extract	Total phenolic content (mg GAE/ g extract)*	Total antioxidant capacity (mg equiv / g BHT)*
Ethanolic	429 \pm 0,1	112, 2 \pm 9,5
Aqueous	368 \pm 0,1	76,6 \pm 2,1
Juice	91 \pm 0,0	82,3 \pm 1,6

*Mean \pm SD.

Hydroxyl free radical-scavenging activity: In figure 3 the juice showed the highest HRSA in a dose-dependent way, being the highest scavenging activity 95.7% at 1 mg/ml. Furthermore, the juice at

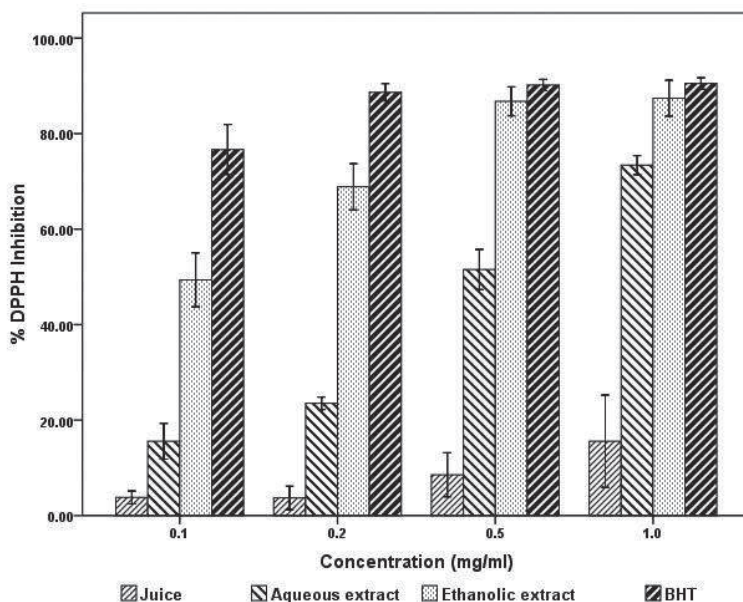


Figure 1. DPPH radical scavenging activity of *P. edulis* extracts.

Data are expressed as mean ± SD. No significant differences were found between the ethanol extract and the positive control ($p = 0.243$) ANOVA, Bonferroni).

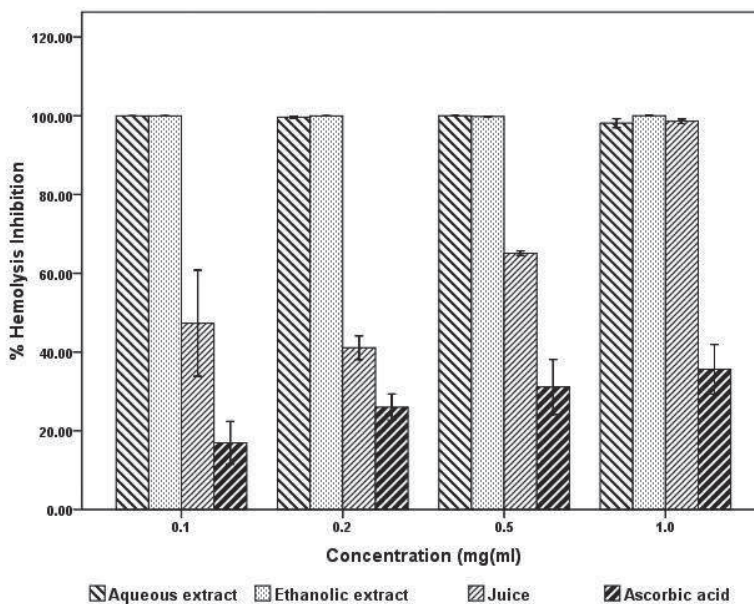


Figure 2. Inhibitory effect of *P. edulis* extracts against hemolysis in human erythrocytes. Data are expressed as mean ± SD. All extracts showed significant differences from control ($p < 0.001$) ANOVA; Bonferroni).

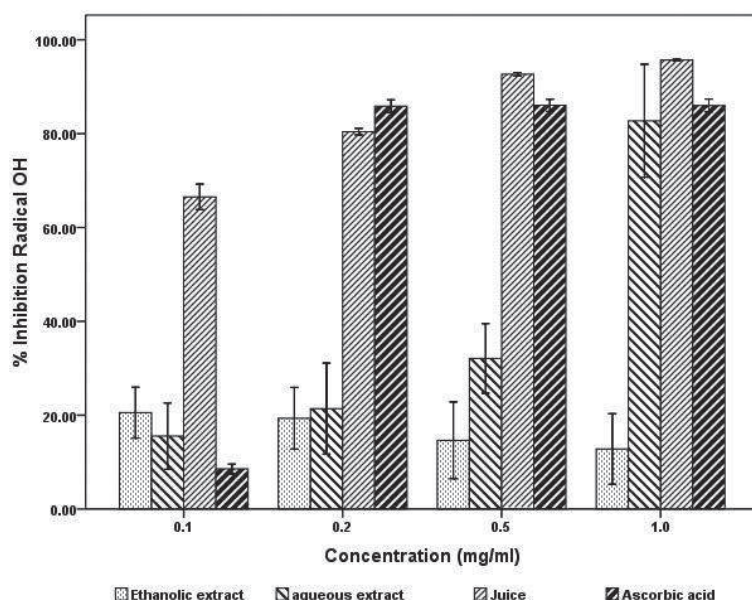


Figure 3. Percentage of HRSA of *P. edulis* extracts. Data are expressed as mean \pm SD. All extracts showed significant differences from control ($p < 0.015$) ANOVA; Bonferroni).

0.5 mg/ml and 1 mg/ml showed HRSA higher than ascorbic acid (positive control); in this study, all the extracts showed significant differences with respect to control ($p = 0.015$). The HRSA of aqueous extract was 82.7% at 1 mg/ml; whereas the ethanolic extract was 20.5% at 0.1 mg/ml, where the HRSA decreased as well as ethanolic extract concentration increased. The EC_{50} for juice was 0.22 mg/ml and for aqueous extract was 0.835 mg/ml.

Total antioxidant capacity: Results presented in table 2 show that ethanolic extract presented the highest total antioxidant activity, 1.46 and 1.36 fold increase compared to the aqueous extract and juice, respectively.

Effect of on SW480 and SW620 cell viability and cell growth: Cytotoxic and antiproliferative activities were evaluated in order to elucidate if the effect of extracts is directly correlated to the

induction of cell death or to the suppression of cell proliferation. The effect of *P. edulis* extracts on SW480 and SW620 cells viability was investigated using MTT assay. As shown in figure 4 the viability effect on SW480 and SW620 decreased as the concentration of each extract also increased from 50 to 500 μ g/ml. This effect was similar in both cell lines and no significant difference was found between treatments in each cell line compared to control (non-treated cells). The IC_{50} value in SW480 cells for ethanolic extract was 524 μ g/ml for aqueous and 444 μ g/ml for juice. In SW620 cells, IC_{50} value for ethanolic, aqueous extracts and juice was 442 μ g/ml, 340 μ g/ml and 415 μ g/ml respectively.

The effect of all extracts on SW480 and SW620 cell growth is presented in figure 5, as OD at 490 nm treated or not. The OD in SW480 cell proteins (Figure 5A) was reduced by 30% and 40%

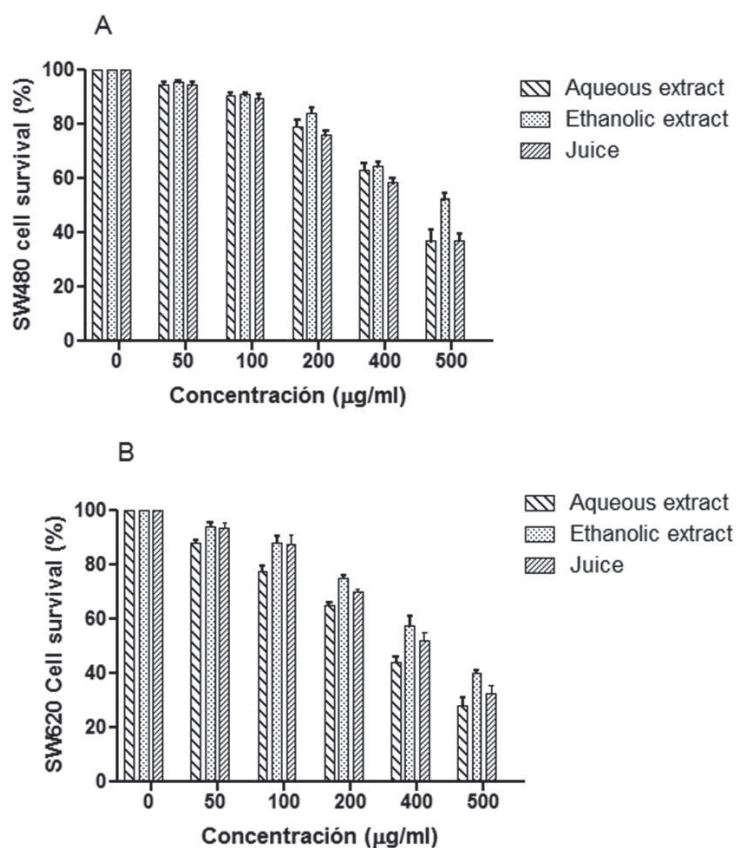


Figure 4. Effect of *P. edulis* extracts on SW480 (A) and SW620 (B) cell viability. Data are expressed as mean \pm SD (n =3, P < 0.05). No significant differences were found between the extracts compared to non-treated cells (DMEM+DMSO) (SW480: p = 0.746; SW620: p = 0.824) ANOVA).

with ethanolic and aqueous and juice extracts, respectively. In SW620 cells, OD was reduced by 60, 50 and 48% after treatment with aqueous, juice and ethanolic extracts, respectively. However, no statistical difference was observed between SW480 (p = 0.753) and SW620 (p = 0.795) cells treated with each extract compared to the respective non-treated cells (DMSO 0.01%).

DISCUSSION

In recent years, biomedical interest in flavonoids and tannins present in plant-derived material has

increased because of their antioxidant properties applied in the prevention of certain oxidative-stress associated diseases such as cardiovascular, cancer, neurodegenerative, and diabetes. The antioxidant property of flavonoids is attributed to phenolic rings that are able to accept an electron to form relatively stable phenoxyl radicals which protects the cell from damage caused by ROS (21).

In the preliminary phytochemical screening of *P. edulis* extracts, the presence of tannins and flavonoids were found, which may be the compounds responsible for the antioxidant activity

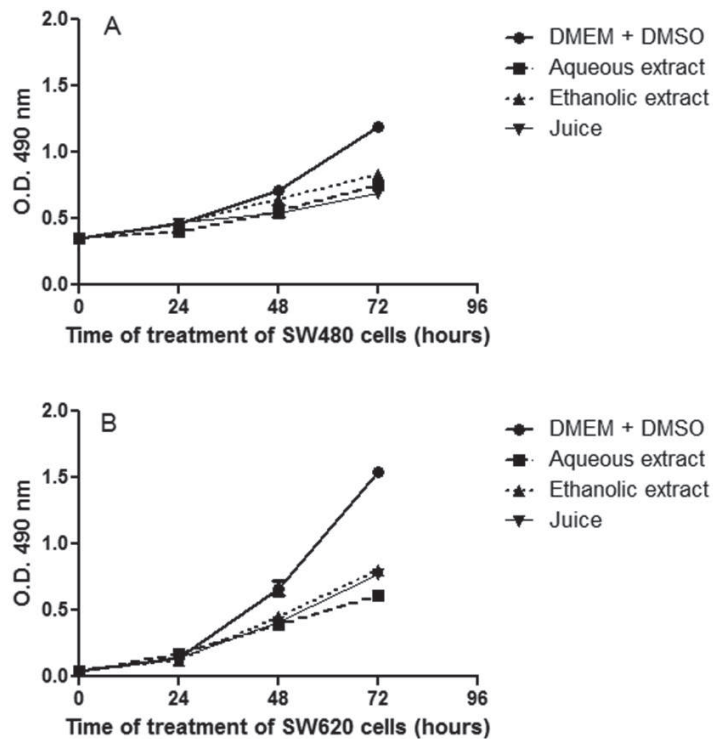


Figure 5. Effect of *P. edulis* extracts on SW480 (A) and SW620 (B) cell growth. Data are expressed as mean \pm SD (n =3, P < 0.05 No significant differences were found between the extracts compared to non-treated cells (DMEM+DMSO) (SW480: p = 0.753; SW620: p = 0.795 ANOVA).

described here because of its well-known ability for scavenging ROS (3). The DPPH assay suggests a direct interaction between ROS and phytochemicals present in *P. edulis* extracts due to the high level energy and kinetic instability of ROS. This is not only attributed to the blocking action of polyphenols but by interacting with their precursors such as superoxide anion and hydrogen peroxide which lead to the hydroxyl radical synthesis which is considered the most potent oxidant.

In this study, *P. edulis* extracts showed an important antioxidant activity observed even at relatively low concentrations and higher than ascorbic acid, which is considered one of the best known

antioxidants. The DPPH radical scavenging assay showed an important antioxidant activity of ethanolic extract (EC_{50} : 0.096 mg/ml) indicating inhibition at the lowest concentration (0.1 mg/ml) more than 50% of DPPH radical. This may be attributed to the presence of flavonoids and quinones in the extract that are able to transfer electrons to DPPH radical and stabilize it, consequently inhibiting their oxidant action. This EC_{50} value is better than one reported by Sunitha and Devaki (22) (EC_{50} = 0.875 mg/ml) and Vasco et al. (23) for ethanolic extract of leaves from *P. edulis* using the same antioxidant activity test, being the highest value of inhibition of DPPH radical 58.17% (22) and 6% at 1 mg/ml (23) indicating a low scavenging efficiency. The EC_{50} value closest

to the result reported here for the antioxidant activity of ethanolic extract of leaves from *P. edulis* was presented by Rojas (21), 0.124 mg/ml. On the other hand, Ripa et al. (24) showed a higher antioxidant activity for this plant, however the solvents used to obtain the extracts are cytotoxic, this is an aspect to be considered for possible therapeutic applications; these discrepancies between the studies may be due to differences in the concentrations of the secondary metabolites in each of the extracts, originated in organic and mineral composition of the soil in which the plants were grown (25).

On other hand, phenolic compounds have also been identified as antioxidants by their ability to inhibit lipid-oxidation processes (26). In our study, the total phenolic content of the ethanolic extract (0.429 mg gallic acid/mg extract) was higher to that described by Rodriguez et al. (27) (phenolic 0.35 mg gallic acid/g extract) and Vasco et al. (23) (0.061 mg GAE/g extract) in methanolic extract indicating a low scavenging efficiency. Moreover, the correlation between the ability of each extract to inhibit DPPH radical with their respective total phenolic content was directly proportional ($p = 0.009$, $r^2 = 0.9998$) and a similar finding was reported by Rudnicki et al. (28) in methanol extracts of leaves from *P. edulis*.

Although passion fruit juice showed DPPH and antihemolytic activities, the total phenolic content and total antioxidant capacity was the lowest compared to the other extracts. However the juice showed the highest HRSA ($EC_{50} = 0.022$ mg/ml and 95.3% inhibition) which could be attributed to the presence of sterols in juice but not in ethanolic and aqueous extracts, which have an important affinity and specificity to scavenge hydroxyl radicals compared to flavonoids and tannins (28). A similar result was reported by Murcia et al. (29) who found that the passion fruit juice inhibits

98.9% hydroxyl free radical, whereas Ferreres et al. (30) found an $EC_{50} > 7$ mg/ml value for HRSA in methanolic extract of leaves from *P. edulis*.

Related to the antihemolytic activity of *P. edulis* there are not previous reports, although some studies with extracts from *Mangifera indica* are able to inhibit 81.7% hemolysis (31) this was lower what was observed here with all *P. edulis* extracts (antihemolytic activity $> 98\%$). Moreover, α -tocopherol was reported to inhibit hemolytic activity by 20.7% at 0.1 mg/ml (32). These results could support future pre-clinical studies that evaluate the antihemolytic activity of these extracts against cardiovascular disease, anemia or medical procedures such as hemodialysis in chronic kidney disease.

In regards to the cytotoxic and antiproliferative activities of *P. edulis* extracts, they presented a dose-dependent response in human colon adenocarcinoma SW480 cells and their derived metastatic SW620 cells. The highest inhibition on SW480 and SW620 cell proliferation was observed with the aqueous extract from leaves, followed by the juice and ethanolic extracts (340 – 524 μ g/ml) which suggests that the extracts contain compounds that may confer antiproliferative activity. However, the National Cancer Institute (NCI) in the United States uses if an antiproliferative extract criterion of $IC_{50} \leq 30$ μ g/ml for cancer cells (33). Using NCI criterion, this suggests that the extracts evaluated here present low activity. In accordance to our study, de Neira (34) found that different fractions of *P. edulis* juice (whole juice, ethanol fraction, carotenoids and polyphenols hydro soluble fraction) inhibited growth of human T cell leukemia MOLT-4 and induced cytotoxicity at 185 - 34800 μ g/ml after 72 h of treatment. Also, Silva et al. (35) reported that an aqueous fraction rich in polysaccharides of *P. edulis f. flavicarpa* was able to inhibit by 40.6%-48.7% the

proliferation of human colon adenocarcinoma cell line HCT-8 at concentrations above 100 µg/ml. Thus, taking together these data suggest that the antiproliferative and cytotoxic effect of extracts of *P. edulis* at concentrations greater than 100 µg/ml could be a specific characteristic of this plant.

The cytotoxic and antiproliferative activities observed in the aqueous and juice extracts might be attributed to the high presence of cardiotoxic glycosides, compared to the ethanolic extract, which have showed antiproliferative and apoptotic effects on various types of cancer cells such as endometrial, ovarian (36), breast (37), leukemia (38), colon, bladder and gastric (39-40), prostate cancer, and glioblastoma (40-41). To confirm this hypothesis, further analyses are required to evaluate the anticancerigen activity of cardiotoxic glycosides-enriched fractions obtained from *P. edulis*.

Several studies on antioxidant activity of extracts rich in polyphenols have shown protective effects against lipid peroxidation. This is of particular importance, because the oxidation of fatty acids of the membrane affects membrane proteins (enzymes, receptors) causing them to lose their functionality; lipid peroxidation appears to have an important role in the pathogenesis of various diseases, especially neurodegenerative and cardiovascular diseases (42).

In conclusion this study showed that ethanolic and aqueous extracts of *P. edulis* present a considerable antioxidant activity even at low concentrations which suggest that daily consumption of passion fruit could be an effective substitute for ascorbic acid and to achieve an antioxidant effect as obtained in this study. Respect to the effect on viability and cell growth on colon cancer cells, the highest activity inhibition on cell proliferation was observed using the aqueous extract and juice, which may be candidates for further analysis with polyphenols and/or cardiotoxic glycosides-enriched fractions to determine their anticancer activity and useful for chemoprevention of this disease .

CONFLICTS OF INTEREST

The authors declare that they did not incur in any conflict of interest during the present study.

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