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Identification of potential bioactive compounds of *Passiflora edulis* leaf extract against colon adenocarcinoma cells

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ABSTRACT

Plant bioactive compounds such as flavonoids and triterpenes can affect lipid metabolism. Here, we report the cytotoxic and lipid-lowering activities of the ethanolic extract of *P. edulis* leaves on human colon adenocarcinoma SW480 cells, also the molecular interactions of bioactive compounds present in *P. edulis* extract on ACC and HMGCR enzymes. The extract reduced cell viability and decreased intracellular triglyceride content by up to 35% and 28% at 24 and 48 h, respectively; whereas the effect was evident on cholesterol only at 24 h. In-silico analysis revealed that luteolin, chlorogenic acid, moupinamide, isoorientin, glucosyl passionflower, cyclopasifloic acid E and saponarin had optimal molecular coupling on Acetyl-CoA Carboxylase 1 and 2 as well as 3-hydroxy-3-methyl-glutaryl-CoA reductase, with possible inhibitory effects. These results show the ability of ethanolic extract to reduce intracellular levels of cholesterol and triglycerides in SW480 cells, which attracts attention for the treatment of colorectal cancer.

1. Introduction

The rapid growth and expansion of tumor tissue often leads to poor and aberrant blood supply, resulting in hypoxia and limited nutrient supply. To thrive in these changing and challenging conditions, cancer cells adapt their metabolism, including that of lipids. Thus, dysregulation of lipid metabolism in cancer involves multiple aspects, including increased lipid uptake, de novo endogenous fatty acid synthesis, fatty acid oxidation, and cholesterol accumulation (important for membrane synthesis and signaling molecules), which promotes tumor growth and progression [1]. Such changes involve the overexpression of key enzymes like Acetyl-CoA Carboxylase (ACC) and 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), promoting triglyceride and cholesterol synthesis, respectively, which are associated with poor prognosis by favoring cancer cell proliferation and migration [1,2]. The irreversible carboxylation of acetyl-CoA to malonyl-CoA carried out by ACC is the rate-limiting step in de novo lipogenesis and therefore a candidate therapeutic [3]. In addition, preventing the active metabolism of cholesterol, for example by inhibiting the MVA pathway, has been shown to be a feasible antitumor strategy [4].

Colorectal cancer is one of the most dangerous forms of cancer, with the potential to spread to different parts of the body, including the liver, lungs, ovaries, and other gastrointestinal organs. Each year, more than 240,000 new cases and approximately 112,000 deaths due to this disease occur in the region of the Americas. Despite advances in technology for diagnosis and treatment [5], its incidence continues to increase and if no action is taken in this regard, it is expected that, by the year 2030, the incidence of colorectal cancer will increase by 60% [6].

The ethanolic extract (EE) of *Passiflora edulis* leaves is a phytochemical mixture that reduces the viability of colon adenocarcinoma SW480 and CaCo2 cells through activation of caspases 3 and 7 [7,8], whereas in hepatocarcinoma HepG2 cells reduce intracellular concentration of fatty acids even in presence of oleic acid [9].

Thus, we evaluated the effect of EE of *P. edulis* leaves on the intracellular concentration of total triglycerides and cholesterol in SW480 by in-vitro analysis, and we determined molecular interactions of bioactive

Abbreviations: ACC, Acetyl-CoA carboxylase; CC, colorectal cáncer; CT, total cholesterol; EE, ethanolic extract; FA, fatty acid; FASN, Fatty Acid Synthase; HMGCR 3, hydroxy-3-methyl-glutaryl-CoA reductase; HPLC-DAD, high performance liquid chromatography-Diode-Array Detection; TG, total triglyceride.

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compounds present on ACC and HMGCR enzymes by in-silico analysis. The above provides an overview of the mechanisms of action of EE on colon adenocarcinoma cells involved in the lipid-lowering and cytotoxic effect.

2. Materials and methods

2.1. Chemicals and instruments

The solvents, chemicals, and reagents used to obtain the plant extract, lipid extraction, and cell viability were of analytical grade and purchased from Merck (Darmstadt, Germany) while all other chemicals/reagents or kits are used for quantifying triglycerides and cholesterol were of analytical grade and were purchased from HUMAN Diagnostics (Wiesbaden, Alemania).

2.2. Extraction procedure

The leaves were collected in the rural township of Tierra Blanca (Valle del Cauca, Colombia) and identified as *Passiflora edulis* (bar code: 18063). Leaves were whased and dried at 37 °C, pulverized using a mill and leached for eight days using ethanol at 96% (the leachate was recirculated constantly). Chlorophylls were removed by liquid-liquid extraction with water and ethanol, the ethanol was evaporated at reduced pressure (60 mbar) and temperature <30 °C in a rotary evaporator, after freeze-dried (Telstar LyoQuest) and powder leaf ethanolic extract (EE) was stored and protected from light at −20 °C until use.

2.3. Phytochemical analysis of the ethanolic extract of Passiflora edulis leaves

Total phenol content was determined by using the Folin Ciocalteu reagent method [10] and results were expressed as mg equivalents of Gallic acid per gram of dry extract (mg DEG/g DS). Total polysaccharide content was determined by using the phenol-sulfuric acid method [11] and results were expressed as mg equivalents of glucose per gram of dry extract (mg EG/g DS). Total tannin and flavonoid content were analyzed as described Armentano et al. [12], and the results were expressed as mg equivalents of tannic acid per gram of dry extract (mg TAE/g DS) and mg equivalents of catechin per gram of dry extract (mg EC/g DS), respectively. The total alkaloid content was determined by using the bromocresol green method [13] and results were expressed as mg equivalents of quinine per gram of dry extract (mg EQ/g DS).

2.4. Cell culture and treatment

SW480 cells were obtained from the American Type Culture Collection (ATCC Manassas, USA), cells isolated from the large intestine of a Dukes C colorectal cancer patient. HFF cells (cell control) were also obtained from ATCC and are human foreskin fibroblasts. Cell maintenance was performed in Dulbecco's Modified Eagle Medium containing 25 mM glucose and 2 mM L-glutamine, 10% heat-inactivated bovine serum (56 °C), 100 U/ml penicillin, 100 μg/ml streptomycin and 1% non-essential amino acids. acids (Merck, USA). The treatment of the cells with the extracts of P. edulis, they were carried out at 37 °C in a humidified atmosphere with 5% CO2, the fetal bovine serum was reduced to 3% and the medium was supplemented with 10 μg/mL of insulin, 5 μg/mL transferrin, 5 ng/mL, selenium (ITS defined medium, Merck, USA).Cells were exposed to DE 24 h after seeding in the cell culture plates and incubated for 24 and 48 h. For cell viability assay, cells were treated with EE at 50, 100, 200, 500, 1000, 2000, and 3000 μg/ml. To determine the effect on intracellular levels of triglycerides and cholesterol, cells were treated with EE at 50, 100, and 200 μg/mL, the concentrations were changed in this last assay to avoid cell death during the incubation periods.

2.5. Cell viability assay

The assay was conducted according to Vichai et al. [14]. Cell lines was cultured in 96-well plates at a density of 1×10^4 cells/mL, treatment was interrupted with 100 μL cold Trichloroacetic Acid at 15% (v/v), incubated at 4 °C for 1 h in darkness, the acid was removed, rapid washes were carried out with water and left to dry at RT. Sulforhodamine B (SRB) was added (0.4% p/v diluted in acetic acid 1%) 100 μL/well and incubated for 30 min at RT, washed with acetic acid (1% v/v in distilled water) and dried overnight, 200 μL Tris-HCl buffer (10 mM pH10.5) was added and optical density was analyzed at 490 nm. The percentage of viability was determined as follow: cell viability (%) = $(AB_t/AB_c)*100$ were AB_T is Absorbance of treatments, AB_C is Absorbance of negative control (untreated cells). The half maximal inhibitory concentration (IC50) was reported for each treatment by line regression.

2.6. Determination of total triglyceride (TG) and cholesterol (CT)

The cells were cultured in six-well plates at a density of 7×10^5 cells/mL, after treatments total cholesterol and triglyceride levels were quantified intracellularly (cell lysate) using Human's liquid color commercial kit (REF: 10017; 10720P) for each lipid following the manufacturer's indications. Additionally, assays were normalized against the total protein.

Statistical analysis. Values represent the mean obtained in three independent assays each in triplicate, data are presented in means ± standard deviation. Data on cell viability were analyzed through one- and two-factor ANOVA and a Tukey test for multiple comparisons through the GraphPad Prism 6.0 statistical software (GraphPad Software Inc., San Diego, CA). The mean separation test between treatments was conducted through Duncan's multiple range test; $p \leq 0.05$ was considered statistically significant.

2.7. In-silico studies

In order to have an approach of which are the possible bioactive compounds of P. edulis that are affecting the total lipid content, it was decided to carry out an in silico molecular docking against the HMGCR, ACC1 and ACC2 targets. The search and selection of compounds was conditional, that is, the compounds used in the molecular docking in this study were based on our previous in vitro investigations [7–9,15] and on other studies [16] that have previously performed the characterization of leaf extracts and antitumor activity in P. edulis.

This study used enzymes ACC1 (code PDB: 3K8X) [17], ACC2 (code PDB: 5KKN) [18], and HMGCR (code PDB: 1DQ9) [19,20] as receptors; each co-crystallized in complex with a native inhibitor ligand B89/tepraloxydim, 6U3/ND-630, and 116/Cerivastatin, respectively. The HMGCR was also cocrystallized with the HMG-CoA catalytic ligand. All X-ray crystallographic structures were obtained from the Protein Data Bank. The BIOVIA Discovery Studio Visualizer 2021 and Autodock Tools programs were used to prepare the three enzyme structures for coupling. Before coupling, non-essential ligands, and cofactors, as well as the water molecules, were eliminated from the protein structure; also, polar hydrogen atoms and Kollman loads were added. The location of amino acids as active sites in the receptor region where the ligand is coupled was determined using Autodock Tools. Targeted coupling was performed on the three enzymes, given the availability of the coordinates of the 3D box due to co-crystallization with the ligand. The Autodock Vina (AV) was used to validate the active site redocking the crystallized native inhibitors on the target receptor to check the coordinates of the active site and the affinity energy of the target-ligand receptor complex. The RMSD value was obtained in triplicate for each enzyme by recoupling the crystallized structure with its ligand and, by comparing it with the "original" crystallized complex, the calculation of the deviation of the two couplings was obtained. It was indicated that the method is valid if the value of the RMSD obtained is ≤ 2 Å [21]. On

the other hand, the search and selection of ligands were conditioned. These compounds were Isoorientine (CAS 4261-42-1), Luteolin (CAS 491-70-3), Saponarin (CAS 20310-89-8), Vitexin (CAS 3681-93-4), Quercetin (CAS 117-39-5), Kaempferol (CAS 520-18-3), Caffeic acid (CAS 331-39-5), Chlorogenic acid (CAS 327-97-9), Gallic acid (CAS 149-91-7), Quinine (CAS 130-95-0), Glucosyl passiflora (CAS 1392-82-1), Cyclopasifloside V (CAS 292167-42-1), Cyclopasifloic acid E (CAS 301540-74-9), Harmane (CAS 486-84-0), Harmol (CAS 487-03-6), and Moupinamide (CAS 66648-43-9). Ligand structures were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) and FoodB (https://foodb.ca/). The Avogadro program was used to add the polar hydrogen atoms, and the MMFF94 force field (molecular force field by Merck) was used to optimize or minimize the energy of the ligand in a vacuum. Thus, the low energy minimums were sought, and it is considered that the ligands are completely flexible, and some receptor atoms (enzymes) are mobile. Using Autodock Tools, Gasteiger charges were added to the compounds. The properties of the compounds were calculated using Lipinski's rule of five (Ro5) performed in the SWISSADME predictor (http://www.swissadme.ch/), Ro5 states that, in general, an orally active drug has no more than one violation of the following criteria: No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds) No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms). Upon validating the active site of the receptors and the flexible plant compounds, molecular docking was carried out using Autodock Vina (AV).

3. Results

3.1. Phytochemical analysis of the ethanolic extract of Passiflora edulis leaves

Table 1 presents the total content of phytochemicals groups of EE. Phenolic compounds represent the largest group quantitatively, among which flavonoids represent 76% of the total phenols in the extract; it was also found that the content of polysaccharides is comparable to the phenol group.

3.2. Effect of ethanolic extract on SW480 and HFF cell viability

EE reduced the viability of SW480 cells in a concentration- and time-dependent manner at 24 h (R2: 0.97) and 48 h (R2: 0.97) ($p \leq 0.05$). At 24 h the percentage of viability decreased significantly from the concentration of 2000 μg/mL (72.6%), at both 24 and 48 h the 3000 μg/mL treatment exhibited the greatest decrease in cell viability with 70.4% and 52.2%, respectively (Fig. 1A). Likewise, the effect on cell viability was significantly greater from 1000 μg/ml (52.2%) when the cells were exposed to EE for 48 h. The IC50 values were 4705 μg/mL and 4269 μg/mL at 24 and 48 h respectively in SW480 cells. In HFF cells, the decrease in the percentage of viability was significant ($p \leq 0.05$) from 1000 μg/mL (81.1%) at 24 h. On the other hand, at 48 h the effect on the decrease in the viability percentage was greater compared to 24 h, being significant for treatments higher than 200 μg/mL (87.1%), in addition, the treatment of 3000 μg/mL exhibited the greatest reduction effect on HFF cell viability at 24 h (71.9%) and 48 h (49.6%) (Fig. 1B). The estimated IC50 at 48 h was 3000 μg/mL in HFF cells.

Table 1
Phytochemical composition of ethanolic extract.

Type of compound	Total compound content
Phenols[1]	239.3 ± 41.0
Polysaccharides[2]	221.9 ± 16.5
Flavonoids[3]	182.4 ± 7.9
Tannins[4]	45.1 ± 3.6
Alkaloids[5]	8.4 ± 1.1

Data are mean ± standard deviation of at least three separate experiments.[1] mg DEG/g DS; [2] mg EG/g DS; [3]mg TAE/g DS; [4] mg EC/g DS; [5]mg EQ/g DS.

3.3. Study of the in-vitro lipid-lowering effect of the EE

Fig. 2A shows the effect of EE on intracellular cholesterol content after 24 and 48 h of treatment. The decrease in cholesterol was significant at 100 μg/mL (75.7 μg cholesterol/μg protein) and 200 μg/mL (65.6 μg cholesterol/μg protein) in SW480 cells at 24 h of treatment compared to untreated cells (89.5 μg cholesterol/μg protein); while in the same conditions, the decrease in triglycerides was significant in all treatments in SW480 cells with respect to untreated cells at 24 h. The 200 μg/mL concentration of EE was the most effective in reducing cholesterol and triglycerides by 27.5% and 35%, respectively in SW480 cells at 24 h of treatment (Fig. 2B) (Table S1).

Fig. 2A, shows the effect of EE on the intracellular cholesterol of SW480 cells after 48 h of treatment at different concentrations. The extract reduced the cholesterol levels compared to the results observed at 24h but this effect did not differ significantly between the treatments at 48 h. However, the intracellular content of triglycerides after 48 h of treatment was reduced significantly at 100 μg/mL (9,0 μg de triglycerides/μg protein) and 200 μg/mL (8,0 μg de triglycerides/μg de protein) compared to untreated cells (11,1 μg triglycerides/μg protein) (Fig. 2B). Thus at 48 h was shown that SW480 treated cells with 200 μg/mL contained 76,2% and 72% total intracellular cholesterol and triglycerides levels respectively, respect to the control group (100%); it means a reduction by 23,8% and 28% in total cholesterol and triglycerides of SW480 treated with EE at 48 h. Taking together these results suggest that EE is able to reduce both lipids in the first 24 h of treatment ($p < 0,05$), affecting more the intracellular content of triglycerides than cholesterol in the SW480 cells (Table S1).

3.4. In-silico study

Table 2 presents the Ro5 for *P. edulis* compounds presents in the ethanolic extract. The isoorientine, luteolin, saponarin, glucosyl passiflora, cyclopasifloside V, and cyclopasifloic ccid E do not comply with the Ro5 because two or more violations. In Table 3, the position of the three native ligands from the crystallography compared to the position of the molecular recoupling shows a differential RMSD for the three ligands <2 Å, which validates the molecular docking site of test compounds in the same box grid area.

Table 4 shows the results of molecular docking analysis between the compounds selected with HMGCR, ACC1 and ACC2 enzymes. Docking results show that luteolin, glucosyl passionflower, cyclopasifloside V, cyclopasifloic acid E and moupinamide had better binding positions with the HMGCR enzyme than HMG-CoA substrate because they had binding free energy values < −9 ΔG. Other compounds were better positioned (binding free energy < −7 ΔG) than native inhibitor 116 Cerivastatin to HMGCR. In addition, binding energy positions with ACC1 which were better or equal to native inhibitor B89/tepraloxydim, were compounds isoorientin, luteolin, saponarin, vitexin, chlorogenic acid, quinine, glucosyl passiflora, cyclopasifloside V, cyclopasifloic acid E, and moupinamide (binding free energy < −6.3 ΔG). And finally, no ACC2 binding positions that were better or equal to native inhibitor 6U3/ND-630 binding positions were found; positions near the free energy of binding of the native inhibitor (−11.7 kcal/mol) were evidenced as glucosyl passiflora and cyclopasifloic acid E at −11 and −10.2 kcal/mol, respectively.

Molecular docking of compounds on the HMGCR enzyme revealed three bioactive compounds with a negative binding to the enzyme that outperformed the native inhibitor, these compounds were luteolin (−9.3 kcal/mol), moupinamide (−9.3 kcal/mol) and chlorogenic acid (−8.3 kcal/mol). Table S2 shows the validation in a 2D diagram with the types of contacts formed between the HMGCR enzyme, its native inhibitor and the compounds luteolin, moupinamide and chlorogenic acid. The interaction between the HMGCR and luteolin, moupinamide and

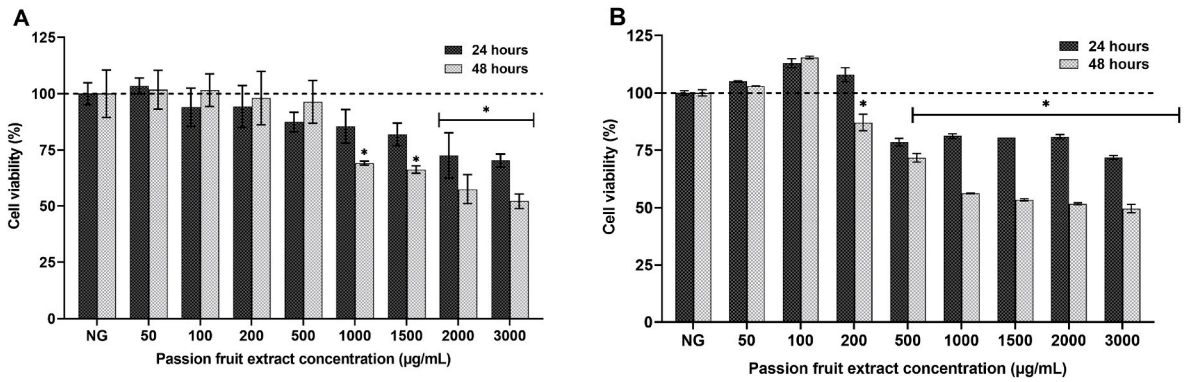


Fig. 1. Effect of ethanolic extract of *Passiflora edulis* leaves on the cell viability of SW480 (A) and HFF (B) at 24 and 48 h at different concentrations. Significant statistical differences exist between the negative control (NG, non-treated cells) and treated cells: *$p \leq 0.05$. Data are presented as mean ± SEM of three independent experiments in triplicate.

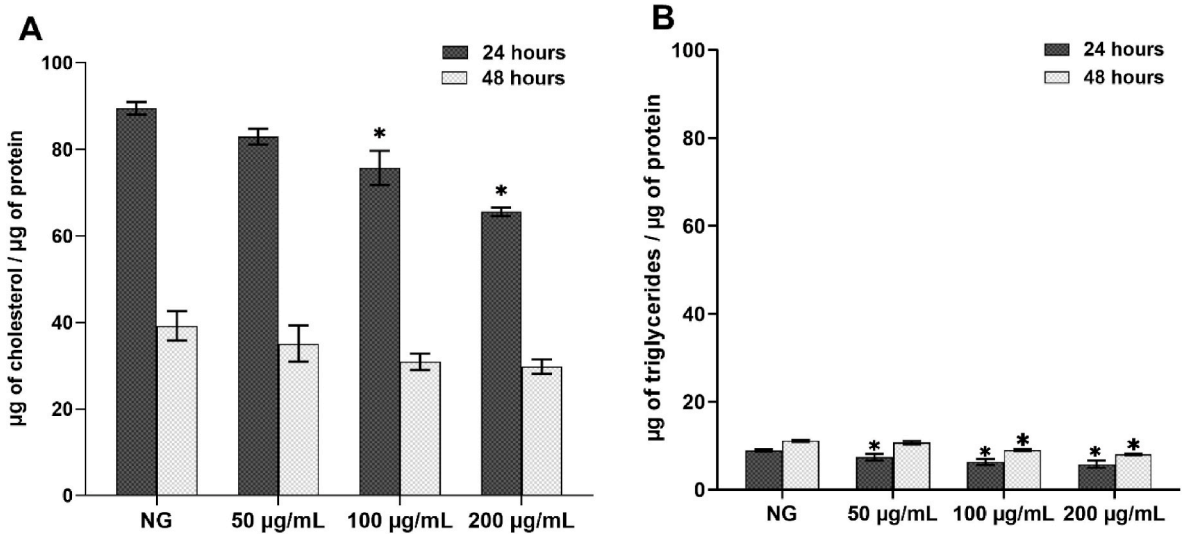


Fig. 2. Effect of ethanolic extract of *Passiflora edulis* leaves on the intracellular content of total cholesterol (A) and triglycerides (B) in SW480 cells at 24 and 48 h. Significant statistical difference between the negative control (NG) and treatments: *$p \leq 0.05$. Data are presented as mean ± SEM of three independent experiments in triplicate.

chlorogenic acid has more stabilizing interactions than HMG-CoA, its native substrate. Luteoline is stabilized by five hydrogen bonds, two hydrophobic bonds and one Pi-donor hydrogen bond; followed by chlorogenic acid with five hydrogen bonds, two hydrophobic bonds and one Pi-alkyl bond; finally, moupinamide with one hydrogen bond, three alkyl bonds and one hydrophobic bond.

Molecular docking analysis for compounds on the ACC1 enzyme showed that, of all the bioactive compounds with binding free energy more negative than the native inhibitor B89/tepraloxydim (Table S3) three were found to have different affinities for the enzyme that bind specifically at the same position as the inhibitor. These were Glucosyl passionflower (−8.6 kcal/mol), Luteolin (−8.0 kcal/mol) and cyclopasifloic acid E (−7.9 kcal/mol). The interaction of the ACC1 enzyme and its native inhibitor B89 occurs through hydrogen bonding, one alkyl, and one Pi-alkyl, as well as Van der Waals interactions. Regarding the three optimal compounds and candidates, luteolin generates more stabilizing interactions with five hydrogen bonds, one hydrophobic and one Pi-alkyl, among others; followed by Glucosyl Passiflora with three hydrogen bonds, one of them being a Pi-donor hydrogen bond; finally, cyclopasifloic acid E with one hydrogen bond, one alkyl, and one hydrophobic bond (Table S3).

Molecular docking analysis for passiflora compounds on the ACC2 enzyme revealed that none of the studied bioactive compounds have a free binding energy higher than the native inhibitor 6U3/ND-30 (Table S3); we identified two compounds with different affinities for the enzyme with binding energy close to the native inhibitor, Glucosyl Passiflora (- 11 kcal/mol) and cyclopasifloic acid E (- 10.2 kcal/mol). Table S3 shows validation of the contacts between ACC2 and the native inhibitor 6U3. Interaction of the complex is stabilized by two hydrogen bonds, one of them is a Pi-donor hydrogen bond, one hydrophobic, and six Pi-alkyl and Van der Waals interaction. The glucosyl pasiflorate compound shows more stabilizing interactions involving eight hydrogen bonds, while E cyclopasifloic acid has only three hydrogen bonds with one Pi-donating hydrogen bond (Table S4).

4. Discussion

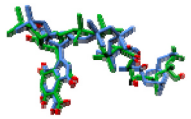
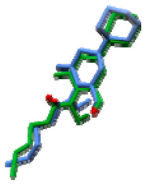
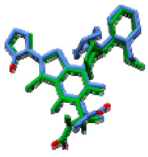
In the present study EE of *P. edulis* leafs reduced viability of colon adenocarcinoma SW480 cells. The total content of cholesterol and triglycerides was evaluated at intracellular levels to deduce a possible mechanism involved in the cytotoxic activity by which in-silico study was performed. We observed that EE decreases the intracellular content of triglycerides and cholesterol at 24 and 48 h of treatment of SW480 cells, involving 16 molecules from *P. edulis* EE [15,16].

The EE presented a higher content of phenolic compounds, among which can be found luteolin, a flavonoid capable of reducing lipogenesis

Table 2
Lipinski's rule of five (Ro5) for passiflora ligands.

Ligands	Molecular formula	Properties					
		Molecular weight(<500 g/mol)	Log P (<5)	H-bond donor (<5)	H-bond acceptor (<10)	Infractions	Complies with Ro5 criteria
B89/tepraloxydim	$C_{17}H_{24}ClNO_4$	341.83	2.97	1	5	0	Yes
6U3/ND-30	$C_{28}H_{32}N_4O_7S$	568.64	2.99	1	8	2	No
116/Cerivastatin	$C_{26}H_{36}FNO$	461.57	4.34	3	7	0	Yes
HMG-CoA	$C_{27}H_{44}N_7O_{20}P_3S$	911.66	−3.89	11	23	3	No
Caffeic acid	$C_9H_8O_4$	180.16	0.93	3	4	0	Yes
Chlorogenic acid	$C_{16}H_{18}O_9$	354.31	−0.39	6	9	1	Yes
Isoorientine	$C_{21}H_{20}O_{11}$	448.38	−0.24	8	11	2	No
Kaempferol	$C_{15}H_{10}O_6$	286.23	1.58	4	6	0	Yes
Luteolin	$C_{26}H_{28}O_{15}$	580.49	−1.09	9	15	3	No
Quercetin	$C_{15}H10O_7$	302.24	1.23	5	7	0	Yes
Saponarin	$C_{27}H_{30}O_{15}$	594.52	−1.65	10	15	3	No
Vitexin	$C_{21}H_{20}O_{10}$	432.38	−0.07	7	10	1	Yes
Glucosyl passiflorate	$C_{37}H_{60}O_{12}$	696.87	2.16	8	12	3	No
Cyclopasifloside V	$C_{43}H_{72}O_{17}$	861.02	0.38	12	17	3	No
Cyclopasifloic acid E	$C_{31}H_{52}O_8$	552.74	2.84	7	8	2	No
Harmane	$C_{12}H_{10}N_2$	182.22	2.70	1	1	0	Yes
Harmol	$C_{12}H_{10}N_{2O}$	198.22	2.16	2	2	0	Yes
Moupinamide	$C_{18}H_{19}NO_4$	313.35	2.39	3	4	0	Yes
Gallic acid	$C_7H_6O_5$	170.12	0.21	4	5	0	Yes
Quinine	$C_{20}H_{24}N_2O_2$	324.42	2.81	1	4	0	Yes

Table 3
Ligand superposition based on HMGCR, 6U3, and B89 recoupling process with crystallography (green: the result of crystallography; blue: the result of recoupling).

Receptor			
Control ligands			
	HMGCR	**ACC1**	**ACC2**
	RMSD (Å)		
HMG-CoA	1.582	N/A	N/A
B89	N/A	1.029	N/A
6U3	N/A	N/A	1.418

Note: N/A: Not applicable.

Table 4
Analysis of molecular docking of passiflora and native ligands on HMGCR, ACC1, and ACC2 enzymes.

Ligands	Binding free energy (kcal/mol)		
	HMGCR	ACC1	ACC2
HMG-CoA	- 9.0	N/A	N/A
116	−7.0	N/A	N/A
B89	N/A	- 6.3	N/A
6U3	N/A	N/A	−11.7
Isoorientine	- 8.3	- 6.3	- 9.3
Luteolin	- 9.3	- 8.0	- 8.9
Saponarin	- 8.3	- 7.2	- 9.7
Vitexin	−8.7	- 6.9	- 9.2
Quercetin	- 8.2	−6.2	- 8.5
Kaempferol	- 8.2	- 6.2	- 8.5
Caffeic acid	−7.1	- 5.5	−6.5
Chlorogenic acid	- 8.3	- 6.4	−9.3
Gallic acid	- 6.7	- 4.5	- 5.7
Quinine	- 7.3	- 6.9	- 9.6
Glucosyl passiflorate	−11.4	- 8.6	- 11
Cyclopasifloside V a	- 9.6	- 9.4	- 9.6
Cyclopasifloic acid E	- 9.9	−7.9	- 10.2
Harmane	- 6.6	- 5.7	- 7.9
Harmol	- 6.7	- 5.6	- 7.8
Moupinamide	- 9.3	- 6.5	- 8.9

Note: N/A: Not applicable.

by inhibiting Fatty Acid Synthase (FASN) and ACC in prostate and breast cancer cells [22]. In addition, our findings show that ethanolic extract contains bioactive compounds with cytotoxic capacity including terpenes, carbohydrates, glucosides like "Passiflorine" and "Passicapsin," alkaloids that can be found in different parts of *Passiflora edulis* [15,16,23,24].

The hypolipidemic effect of EE on SW480 cells is comparable to that reported by Sari et al. [25], who evaluated a *P. edulis* seed extract (100 mg and 50 mg extract/kg body weight) in rats for two weeks and also observed significant reduction of serum triglycerides. With these findings, we aimed to find out compounds of passiflora that could be involved in the cholesterol and triglyceride lowering effect on SW480 and thus affect its viability. For this purpose, in-silico methodology was used to identify bioactive compounds antagonistic to the binding site of HMGCR, ACC1 and ACC2 enzymes.

According to Istvan et al. [19,20], the binding site area of HMGCR involves six types of residues (asparagine, tyrosine, alanine, lysine, serine and histidine) at 12 positions in the active site for hydrogen bonds (ASN567, TYR479, ALA564, TYR479, ARG571, LYS722, ARG568, SER852, SER565, HIS866, SER865 and LYS755). By re-coupling the catalytic ligand of HMG-CoA and analyzing the molecular bonds, seven exact overlaps of catalytic site residues (LYS735, SER565, HIS752, ARG568*2, ASN567 and LYS722) were found. For cerivastatin inhibitor 116, of the four mentioned ligands, all are in exact positions for the binding site residues reported in crystallography (SER565, SER865, ARG568, and LYS722) (Table S2). According to Istvan et al. [19], one of the most important positions is Ser565 because a hydrogen bond is formed. Previous studies also reveal how statins bind to HMGCR and inhibit its activity, these are bulky, hydrophobic compounds which occupy the HMG binding pocket and part of the CoA binding surface. Thus, access of the HMG-CoA substrate to HMGCR enzyme is blocked and its tight binding is probably due to Van der Waals interactions. Therefore, passiflora bioactive compounds that bind to the active site of HMGCR enzyme are potential inhibitors. The interactions of compounds with a more negative binding affinity (better binding strength) than that reported through native 116/Cerivastatin inhibitor binding (−7.0

kcal/mol) were 11 compounds. However, due to the overlap of the native inhibitor for the compounds of interest, the best candidates were luteolin, moupinamide, chlorogenic acid.

Xiang et al. [26], reported that the binding site of ACC1 enzyme involves three residues (glycine, isoleucine and alanine) located at four positions in the active site that form hydrogen bonds (GLY1734, IIE1735, ALA1627, GLY1998). In the rearrangement of the native inhibitor and after molecular bond analysis, three exact overlaps of catalytic site residues were found (ALA1627, TYR1738 and ALA1712); however, ALA1627 formed hydrogen bonds, while the remaining two residues formed hydrophobic bonds, which added to Van der Waals interactions gave stability to the complex. Xiang et al. [26] mentioned that TYR1738 residue induces conformational changes by opening a small pocket at the interface of the biotin carboxylase (CT) domain dimer. In comparison, the pocket does not exist in the free structure of the enzyme, underscoring the functional importance of these small conformational changes; without these changes, the ligand cannot bind to the enzyme; therefore, there is no CT enzyme activity. So, this binding site may be an important anchor point for developing CT domain inhibitors. For the overlay of the native inhibitor, the best candidates were luteolin, cyclopasifloic acid E, glucosyl passiflora and saponarin; also, the energy and characteristic position of the compound represented that it bound to more than one residue of the enzyme active site (TYR1738, GLY1734 and ALA1627) via hydrogen bonds, hydrophobic and alkyl bonds (Table S2). Importantly, hydrogen and hydrophobic bonds, such as Pi-Sigma, Pi-alkyl, Alkyl, and Van der Waals interactions in inhibitor binding on ACC1 favor the inhibition.

Compounds glucosyl passiflora (−11 kcal/mol) and cyclopasifloic acid E (−10. 2) exhibited a binding free energy fairly close to those of 6U3/ND-630, they are possibly not potent inhibitors of ACC2, but are postulated as candidates for modulating enzyme activity, because overlap was observed. Further, they had acceptable levels of free energy in comparison with the energetic value of catalytic compounds, about −10 kcal/mol. In Addition, they demonstrated to be linked to more than one residue of the receptor active site (ARG281 and TRP681) by hydrogen bond interactions, hydrophobic bonding and Pi-donating hydrogen bonding, two of the residues and positions correspond to the 6U3 binding site in ACC2 through hydrogen and hydrophobic bonds [18].

The ACC enzyme exist as two tissue specific isoenzymes encoded by separate genes and different distribution in cells [18]. ACC1 is a cytosolic enzyme in lipogenic tissues (liver, adipose tissue), whereas ACC2 is associated with mitochondria of oxidative tissues (liver, heart, skeletal muscle). In liver, the Malonyl-CoA compound produced in cytoplasm by ACC1 is used in the synthesis and elongation of FA. In contrast, Malonyl-CoA formed in mitochondrial matrix by ACC2 enzyme is used for regulating the beta-oxidation of mitochondrial FA by allosteric inhibition of carnitine palmitoyltransferase-1. In this way, the inhibition of FA biosynthesis and/or the stimulation of the oxidation can affect growth cancer cells.

The rule of five (Ro5) is a useful method for determining whether a compound has pharmacological potential. According to this rule, orally active drugs must not be non-compliant with the established criteria [27]. Some of the compounds that showed Ro5 non-compliance are luteolin (3), cyclopasifloic acid E (2), glucosyl passionflower (3), saponarin (3), isoorientin (2); while moupinamide and chlorogenic acid complied with Ro5. The five compounds that failed to comply with the Ro5 can be attributed to low solubility and low membrane permeability; whereas moupinamide and chlorogenic acid have the closest resemblance with a drug in terms of chemical, physical and pharmacological properties.

5. Conclusions

EE contains phenols, polysaccharides, flavonoids, tannins and alkaloids that together have a cytotoxic effect on SW480 cells, furthermore the extract reduced the intracellular concentration of triglycerides and cholesterol in the short term. Seven plant compounds (luteolin, chlorogenic acid, moupinamide, isoorientin, glucosyl passionflower, cyclopasifloic acid E, and saponarin) were identified by *in-silico* analysis as possible antagonists of native substrates of the limiting enzymes of triglyceride and cholesterol biosynthesis, a possible mechanism of EE involved in the cytotoxic effect on SW480 cells. These findings suggest that *Passiflora edulis* leaves are a source of bioactive compounds for the development of therapeutic products against colon adenocarcinoma.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Universidad del Quindio, Universidad de Antioquia reports financial support was provided by University of Antioquia. Maria Elena Maldonado Celis reports a relationship with University of Antioquia that includes: employment. The authors declare that they are not influenced by: 1. economic interests 2. personal interests 3. secondary benefits, and therefore it does not affect this research work. 4. None of the authors have editorial ties with this journal or the Elsevier group, 5. Nor any other benefit other than the dissemination of this new knowledge.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101453.

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