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Phytochemical profile and dual anticancer–antilipase activities of *Rubus ulmifolius* extracts from Jijel, Algeria

Saliha Hireche[1,2], Rachid Belhattab[1,3*], Asma Cherbal[2,4], Violet Kasabri[5], Zeynep Aydoğmuş[6], Sundus H. Al Alawi[5], Fatma U. Afifi[7], Mohammed Kebieche[8] and Abderrachid Desdous[2]

[1]Laboratory of Applied Microbiology, Ferhat Abbas Setif1University, Setif, Algeria. [2]Department of Molecular and Cell Biology, Faculty of Nature and Life Sciences, University of Jijel, Jijel, Algeria. [3]Department of Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas Setif 1University, 19000, Setif, Algeria. [4]Laboratory of Biomathematics, Biophysics, Biochemistry and Scientometry, Faculty of Nature and Life Sciences, University Abderrahman Mira, Béjaïa, Algeria. [5]Faculty of Pharmacy, The University of Jordan, Amman, Jordan. [6]Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey. [7]Faculty of Pharmacy, Applied Science Private University, Amman, Jordan. [8]Faculty of Nature and Life Sciences, University of Batna2, Batna, Algeria. *Author for correspondence. E-mail: rbelhat@yahoo.fr

ABSTRACT. This study evaluated the *in vitro* antiproliferative and antilipase activities of ethanolic and methanolic extracts from *Rubus ulmifolius* (Jijel, Algeria) against obesity-related colorectal cancer (CRC) cell lines (HT29, HCT116, SW620, CACO2, SW480) and primary human fibroblasts. Cells were treated for 72 h and viability was quantified by SRB assay. The ethanolic extract yielded IC_{50} (μg mL^{-1}, mean ± SD) values of 73.3 ± 9.9 (HT29), 106.8 ± 8.5 (HCT116), 240.2 ± 16.4 (SW620), 50.5 ± 5.2 (CACO2), 109.8 ± 3.7 (SW480), and 229.2 ± 16.8 (fibroblasts). The methanolic extract gave 121.2 ± 8.4 (HT29), 102.0 ± 3.9 (HCT116), 147.1 ± 10.3 (SW620), 26.2 ± 1.5 (CACO2), 95.0 ± 9.2 (SW480), and 157.3 ± 8.2 (fibroblasts), with activity in CACO2 meeting the NCI threshold for crude extracts (IC_{50} < 30 μg mL^{-1}). Cisplatin (positive control) showed IC_{50} values of 19.72 ± 1.62 (HT29), 878.12 ± 105.4 (HCT116), 173.8 ± 21.0 (SW620), 175.41 ± 21.05 (CACO2), 26.9 ± 0.061 (SW480), and 1.52 ± 0.18 (fibroblasts), underscoring the relatively lower cytotoxicity of plant extracts toward normal cells. In pancreatic lipase assays, the ethanolic extract was more potent than the methanolic extract (IC_{50} 30.2 ± 1.1 vs. 120.2 ± 8.8 μg mL^{-1}), while orlistat gave 0.11 μg mL^{-1}. HPLC-DAD-UV profiling identified rutin as the predominant phenolic (6.17–7.09 mg100 mg^{-1} extract). GC-MS indicated that the ethanolic extract was enriched in fatty acids (oleic acid 17.62%, Z-6-octadecenoic acid 15.36%, methyl linolenate 12.81%), whereas the methanolic extract was rich in monoterpenoids (carvacrol 35.87%, thymol 21.14%, o-cymene 8.74%). Collectively, these data suggest *R. ulmifolius* contains constituents with dual anti-CRC and antilipase potential and merits further bioassay-guided fractionation.

Keywords: Polyphenols; antiproliferative; cytotoxicity; lipase;

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Introduction

Colorectal cancer (CRC) remains a significant global health concern, ranking as the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths, with approximately 1.4 million new cases and 700,000 deaths reported in 2012 (Arnold et al., 2017). Platinum-based compounds, such as cisplatin, carboplatin, and oxaliplatin, are a cornerstone of chemotherapy for various solid tumors, including CRC (Zhang et al., 2022). While oxaliplatin is part of the standard first-line treatment for CRC, its use is often limited by the development of acute and chronic painful neuropathy (Mauri et al., 2020).

Concurrently, obesity has emerged as a major global health issue, linked to various comorbidities including cardiovascular disorders, musculoskeletal problems, and an increased risk of several cancers, including CRC (Billington et al., 2000). Pancreatic lipase, a key enzyme in the digestion of dietary triglycerides, plays a crucial role in lipid absorption, and its inhibition has been widely studied as a strategy for anti-obesity therapies (Lunagariya et al., 2014). Orlistat, a pancreatic lipase inhibitor, is currently approved for long-term obesity treatment. However, its use is associated with significant gastrointestinal side effects, limiting patient compliance (Kwon et al., 2022).

Given these challenges, there is a pressing need for novel therapeutic agents that can effectively target CRC and/or obesity while minimizing adverse effects. Plant-derived compounds are increasingly recognized as promising candidates for therapeutic development due to their perceived lower toxicity, fewer side effects,

and relatively lower cost compared to synthetic drugs (Ferdjioui et al., 2024). *Rubus* species (family Rosaceae) have a long history of traditional use for various therapeutic purposes (Asnaashari et al., 2015). Specifically, *Rubus ulmifolius* is used in folk medicine, with its leaves and young shoots being utilized for their anti-inflammatory, anti-odontalgic, and gastrointestinal spasmolytic properties. Crushed young shoots are also applied topically to wounds, infected insect bites, and skin eruptions (Sisti et al., 2008). Furthermore, plant polyphenols, including those found in *Rubus* species, have garnered significant attention for their potent antioxidant properties, which have been implicated in the prevention of various diseases associated with oxidative stress, such as cancer, inflammation, and diabetes (Cherbal et al., 2017, 2022, Hireche et al., 2021).

This study aims to explore the potential of *Rubus ulmifolius* extracts to inhibit obesity-related CRC cell growth and pancreatic lipase activity. We evaluated the *in vitro* effects of ethanolic and methanolic extracts of *R. ulmifolius* on a panel of colorectal cancer cell lines. We also analyzed the extracts using HPLC-DAD-UV with phenol standards and GC/MS to identify major components. This research is driven by the hypothesis that natural plant extracts may offer a source of novel compounds for the treatment of CRC, while simultaneously addressing obesity-related factors.

Materials and methods

Reagents

Dulbecco's Modified Eagle Medium (DMEM), high glucose (25 mM) (Invitrogen, Carlsbad, CA, USA; Cat. No. 11965092) was used for cell culture. Fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA; Cat. No. 16000044), penicillin–streptomycin solution (Gibco; Cat. No. 15140122), and trypsin-EDTA (Gibco; Cat. No. 25200056) were also used. Di-n-octyl phthalate standard (Sigma-Aldrich, St. Louis, MO, USA; Cat. No. 36735) and other key analytical standards were purchased from Sigma-Aldrich. Unless otherwise specified, all other reagents and solvents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant extracts

Rubus ulmifolius aerial parts were collected in Jijel, Algeria. The plant material was identified by Dr. Hanane Khennouf from the Department of Environment and Agronomy Sciences at the University of Jijel. For the preparation of the methanolic extract, 80 g of the dried, ground plant material was weighed and macerated in 800 mL of a methanol-water solution (8:2, vv^{-1}). The mixture was stirred manually several times, then left to macerate at room temperature for 24 hours. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator (Heidolph Laborota 2000) at a temperature below 60°C. This extraction process was repeated three times using fresh solvent each time. The ethanolic extract was prepared following the same procedure, substituting methanol with ethanol.

Cell lines and seeding conditions

In this study, human colorectal cancer cell lines HT29, HCT116, SW620, SW480, and CACO2, obtained from the American Type Culture Collection (ATCC), were used, alongside primary human periodontal ligament fibroblasts (PDL) from ScienCell Research (Carlsbad, CA, USA) for selectivity cytotoxicity testing. The CACO2 cell line (ATCC® HTB-37™) is derived from a 72-year-old Caucasian male with a colon adenocarcinoma and expresses epidermal growth factor (EGF). The HCT116 cell line (ATCC® CCL-247™) is derived from a 48-year-old male patient with a Dukes' stage D colorectal carcinoma of the ascending colon. The HT29 cell line (ATCC® HTB-38™) originates from a 44-year-old female patient with a Dukes' stage C colon adenocarcinoma. The SW620 cell line (ATCC® CCL-227™) was derived from a 51-year-old male patient's Dukes' stage C colorectal adenocarcinoma lymph node metastasis. Lastly, the SW480 cell line (ATCC® CCL-228™) comes from a 50-year-old male patient's colon tissue, showing epithelial morphology of Dukes' type B colorectal adenocarcinoma. All cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 25 mM glucose, 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine (2 mM), 50 $IUmL^{-1}$ penicillin, and 50 μgmL^{-1} streptomycin, and incubated at 37°C in a humidified atmosphere containing 5% CO_2. For assays, cells were seeded at a density of 5,000 cells per well in 96-well plates. This seeding density was optimized to provide reliable readings with the sulforhodamine B (SRB) assay, correlate well with cell numbers, and ensure exponential growth during the incubation period with the plant extracts being tested.

Cytotoxicity assay

This assay assessed the cytotoxic effects of extracts and pure compounds on cells. Briefly, cells were prepared as follows: Cells were washed three times with phosphate-buffered saline (PBS). Following PBS removal, cells were detached using 0.025% trypsin-EDTA (Sigma). Dulbecco's Modified Eagle Medium (DMEM) was added to a final volume of 10 mL, and the cell suspension was centrifuged at 1000 x g for 10 minutes. The resulting cell pellet was resuspended in 10 mL of DMEM, producing a single-cell suspension. Cell viability, assessed via trypan blue exclusion using a hemocytometer, was consistently >90%.

The cell suspension was then diluted to the optimal seeding density, and 100 µL was aliquoted into each well of a 96-well plate. Plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours, allowing for cell adherence. Following this incubation, cells were treated with either extracts or pure compounds. Each extract, initially dissolved in dimethyl sulfoxide (DMSO), was diluted in DMEM and filter-sterilized using a 0.2 µm filter prior to use. An initial screening concentration of 50 µg mL^{-1} of each extract was tested. Extracts demonstrating <50% cell survival after 72 hours of exposure were classified as active and selected for further analysis.

These active extracts were subsequently serially diluted in DMEM to create six concentrations (5, 10, 25, 50, 100, and 200 µg mL^{-1}). 100 µL of each concentration was added to the cell-containing wells in triplicate or quadruplicate (independent replicates). Importantly, the final concentration of DMSO in the cell culture media never exceeded 1%, and medium containing 1% DMSO served as the solvent control. The plates were then incubated for 72 hours. Cell growth was quantified at the end of the exposure period using the sulforhodamine B (SRB) assay. Cisplatin, at concentrations ranging from 0.1 to 200 µg mL^{-1}, served as a positive control.

Sulforhodamine B (SRB) assay

After a 72-hour incubation, adherent cell cultures were fixed in situ by the addition of 50 µL of ice-cold 40% (wv^{-1}) trichloroacetic acid (TCA) solution to each well. Plates were incubated at 4°C for 60 minutes. Following fixation, the TCA was removed, and the plates were thoroughly washed five times with deionized water. The plates were then allowed to air-dry completely. Protein was stained by adding 50 µL of 0.4% (wv^{-1}) Sulforhodamine B (SRB) solution in 1% acetic acid to each well, and the plates were incubated for 30 minutes at room temperature. Unbound SRB was removed through five washes with 1% acetic acid, followed by a further air-drying step. Finally, protein-bound dye was solubilized by adding 100 µL of 10 mM Tris base (pH 10.5) to each well. Plates were then shaken gently on a plate shaker for 20 minutes to ensure thorough solubilization. Absorbance was measured at 570 nm using an microplate reader. Cell survival was calculated as the percentage of absorbance of treated samples relative to the absorbance of control (untreated) cells. The half-maximal inhibitory concentration (IC_{50}) was determined by non-linear regression analysis of the dose-response curves (Cherbal et al., 2022).

Assessing pancreatic lipase activity and inhibitory effects of test extracts

Preparation of test solutions

Methanolic and ethanolic extracts were initially dissolved in Tris-HCl buffer (2.5 mM, pH 7.4, containing 2.5 mM NaCl) to create five stock solutions each, with concentrations ranging from 0.01 to 100 mg mL^{-1}. Subsequently, a 20 µL aliquot of each stock solution was added to the reaction mixture, resulting in final assay concentrations ranging from 0.2 to 2000 µg mL^{-1}.

The reference drug, orlistat, was prepared as a 1 mg mL^{-1} stock solution in DMSO. This stock was then used to generate six working stock solutions with concentrations ranging from 0.625 to 20 µg mL^{-1}. A 20 µL aliquot of each orlistat working stock solution was then added to the reaction mixture, yielding final assay concentrations ranging from 0.0125 to 0.4 µg mL^{-1}.

Pancreaticlipase activity assay

In vitro pancreatic lipase (PL) activity was assayed based on the method described by Cherbal et al. (2022), and used to determine the concentration of compound required for 50% inhibition of PL activity (IC_{50}). PL activity was quantified by a colorimetric assay measuring the release of *p*-nitrophenol.

Porcine pancreatic lipase type II (Sigma, USA, EC3.1.1.3) was used as the enzyme source. The enzyme solution was freshly prepared by suspending crude PL in Tris-HCl buffer (2.5 mM, pH 7.4, containing 2.5

mM NaCl) to a concentration of 5 mg mL^{-1} (equivalent to 200 U mL^{-1}). The suspension was mixed using a magnetic stirrer for 15 minutes, then centrifuged at 1500 x g for 10 minutes, and the supernatant was collected.

For the enzymatic assay, 100 µL of the PL solution was pre-incubated with different concentrations of test extracts or orlistat for 5 minutes at 37°C. The reaction was then initiated by adding 5 µL of the *p*-nitrophenyl butyrate (PNPB) substrate (10 mM in acetonitrile). The final reaction volume was adjusted to 1 mL using Tris-HCl buffer. The absorbance of the solution was measured spectrophotometrically at 410 nm, starting 1 minute after substrate addition and continuing at 0.5-minute intervals for an additional 4 minutes.

Pancreatic lipase (PL) activity was quantified by monitoring the release of *p*-nitrophenol at 410 nm and calculating the reaction rate from the slope (ΔA/Δt) of the linear segment of the absorbance–time trace. The inhibitory activity of pancreatic lipase was then estimated using the following formula:

$$\text{Inhibitory activity } \% = \frac{\text{Slope}_{\text{control}} - \text{Slope}_{\text{test}}}{\text{Slope}_{\text{control}}} \text{x } 100$$

where:

Slope control is the slope of the linear segment of the absorbance–time trace for the solvent control (no inhibitor).

Slope test is the slope of the linear segment of the absorbance–time trace for the test extract or orlistat.

HPLC-DAD-UV analyses

Sample preparation

A dry extract stock solution was prepared by dissolving the extract in 80% methanol to a concentration of 20 mg mL^{-1}. This solution was then filtered through a 0.22 µm pore size acetate membrane to remove particulate matter. The filtered stock solution was subsequently diluted with 80% acetonitrile to a working concentration of 10 mg mL^{-1}. Finally, 2 mL aliquots of the diluted sample were transferred to amber glass HPLC vials for analysis by RP-HPLC with a Diode Array Detector (DAD).

Standard preparation

Reference standards for rutin, quercetin, vanillic acid, kaempferol, epicatechin hydrate, caffeic acid, riboflavin, curcumin, coumaric acid, tannic acid, gallic acid, chlorogenic acid, and vanillin were obtained from Sigma-Aldrich (USA). Stock solutions of each standard were prepared at a concentration of 0.5 $mgmL^{-1}$ in methanol. Working standard solutions were prepared by diluting each stock solution with 80% acetonitrile to a final concentration of 10 µg mL^{-1}.

HPLC analysis

Chromatographic analysis was performed using a Thermo-Finnigan Spectra System HPLC instrument (San Jose, CA), consisting of a P4000 pump, SN 4000 system controller, AS3000 autosampler, and a UV 6000 LP detector equipped with a photodiode array. The detection was set to scan from 200 nm to 500 nm. Data acquisition and analysis were performed using ChromQuest 5.0 software. Separations were achieved on a reversed-phase Waters Symmetry C18 column (5 µm particle size, 100 Å pore size, 4.6 × 250 mm; Waters, USA).

A gradient elution was performed using a mobile phase of acetonitrile (solvent A) and 20 mM phosphoric acid (solvent B). The gradient program was as follows:

* 0.00–10.00 min: 5% A to 10% A.
* 10.00–50.00 min: 10% A to 12% A.
* 50.00–70.00 min: 12% A to 15% A.
* 70.00–90.00 min: 15% A to 25% A.
* 90.00–110.00 min: 25% A to 35% A.
* 110.00–120.00 min: 35% A to 30% A.
* 120.00-130.00 min: 30% A.

The mobile phase was degassed prior to injection and pumped at a flow rate of 0.70 mL min^{-1}. The injection volume was 10 µL. The detector was set to monitor the analytes at wavelengths of 265 nm, 330 nm, and 370 nm. The identification of analytes was based on the comparison of their retention times and UV spectra

to those of the corresponding pure standards. Quantification was achieved by comparison with the standard curves obtained from analysis of the reference standards.

GC-MS analysis

GC-MS analysis was performed on a Shimadzu QP2010 mass spectrometer using electron impact ionization (70 eV). A capillary column (0.25 mm × 25 m) coated with 5% diphenyl and 95% dimethyl polysiloxane (SE-30) was used for the separation. The oven temperature was programmed to increase from 50 to 250°C at a rate of 5°C min^{-1}. The transfer line temperature was maintained at 250°C. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1} with a split ratio of 20:1. The mass spectrometer was operated in scan mode, acquiring data from m z^{-1} 40–350 with a scan time of 0.50 s. The identification of components was based on the comparison of their retention times and mass spectra with those in the NIST mass spectral library. The percentage composition was determined based on peak area.

Statistical analysis

Data are presented as mean ± standard deviation (SD) derived from 3-4 independent experiments. Statistical differences between treatment groups and their respective controls were assessed using analysis of variance (ANOVA), followed by either Tukey's or Dunnett's post hoc multiple comparisons test, as appropriate for the experimental design. These analyses were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, California USA). Differences were considered statistically significant at a p-value of < 0.05.

Results

Antiproliferative activity

The *in vitro* effects of *Rubus ulmifolius* ethanolic and methanolic extracts on inhibiting the growth of various tumor cell lines were evaluated. The observed inhibitory potencies varied significantly based on cell type, and the two extracts displayed distinct effects (Figure 1).

IC_{50} values were calculated for each extract and cell line, and the results are summarized in Table 1.

Table 1. IC_{50} values (µg mL^{-1}) for *in vitro* antiproliferative activity of *Rubus umifolius* ethanolic and methanolic extracts and cisplatin in colorectal cancer cell lines.

Treatment	Cytotoxicity (as of % Control) IC_{50} value µg mL^{-1}					
	HT29	HCT116	SW620	CACO2	SW480	Fibroblasts
Rubus ethanol extract	73.3±9.9	106.8±8.5 [i]	240.2±16.4 [a]	50.5±5.2[iii]	109.8±3.7 [iv]	229.2±16.8[a]
Rubus methanol extract	121.2±8.4 [b]	102±3.9 [i]	147.1±10.3 [b, ii]	26.2±1.5[iii]	95±9.2 [iv]	157.3±8.2 [b]
Cisplatin	19.72±1.62[c]	878.12±105.4	173.8±21 [ii]	175.41±21.05	26.9±0.061[c]	1.52±0.18 [c]

Data are presented as mean ± SD (n = 3-4 independent replicates). Within each row, treatment group means were compared to the control (fibroblast) mean using two-way ANOVA followed by Dunnett's post hoc multiple comparisons test. Values in a row not significantly different from the fibroblast control are labeled with the same letter (a-c). Within each column, treatment group means were compared to each other using two-way ANOVA followed by Tukey's post hoc multiple comparisons test. Values within a column not significantly different from each other are labeled with the same Roman numeral (i-iv).

The study revealed a diverse range of responses among colorectal cancer cell lines to*Rubusulmifolius*extracts and cisplatin. The IC_{50} values, reflecting the potency of each treatment, showed considerable variation among the different cell lines tested, highlighting their differing sensitivity to these treatments. As expected, cisplatin was found to be the most potent in most of the cells tested, especially in the HT29 cell line, where it displayed the lowest IC_{50} value (19.72±1.62); this is in agreement with its known use as a chemotherapeutic drug. However, the *R. ulmifolius* extracts, both ethanolic and methanolic, were generally less potent than cisplatin, with higher IC_{50} values (ranging from 26.2±1.5 to240.2±16.4), which is to be expected of a crude extract. Importantly, it is noted that the *R. ulmifolius* extracts were considerably less potent on the fibroblasts, showing selectivity for the tumor cells, whereas cisplatin was very potent in both tumor and fibroblasts cell lines, suggesting it is not selective for tumor cells. Further examination also revealed differences between the effects of the methanolic and ethanolic extracts; the methanolic extract showed higher activity in the CACO2 and SW480 cells, while the ethanolic extract was more potent in the CACO2 and HT29 cells, thus highlighting the role that solvent polarity plays in the activity of the extract.

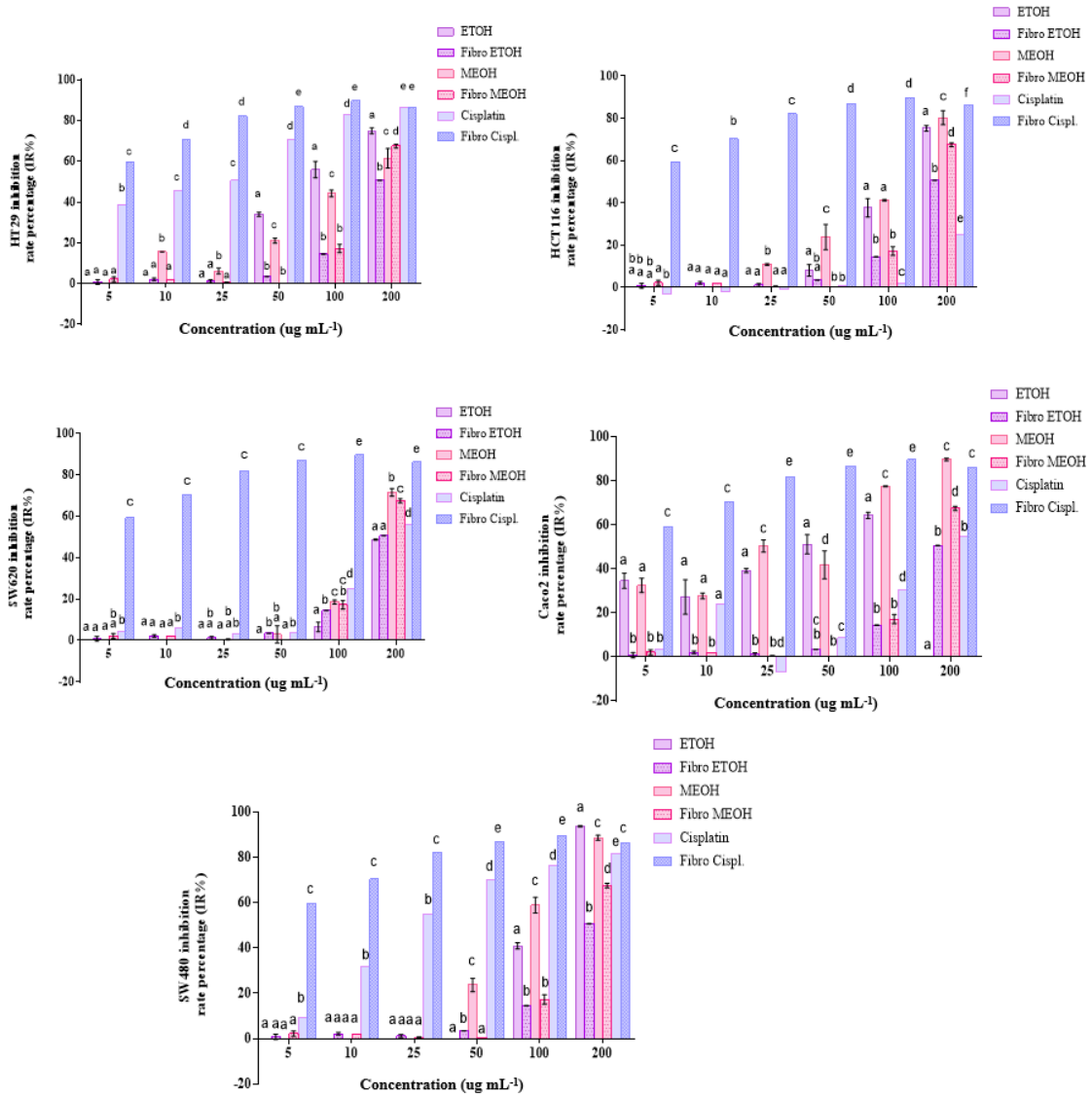

Figure 1. *In vitro* cytotoxic activity of *Rubus ulmifolius* ethanolic and methanolic extracts against colorectal cancer cell lines (HT29, HCT116, SW620, CACO2, and SW480). Data are presented as mean ± SD (n=3). Statistical significance was determined using Tukey's multiple comparisons test, with comparisons made between all groups at each concentration. Bars labeled with the same letter indicate no statistically significant difference ($p \geq 0.05$).

Pancreatic lipase inhibition activity

The *in vitro* assessment of pancreatic lipase inhibition revealed differences in activity between the *Rubus ulmifolius* extracts. Notably, the ethanolic extract exhibited a higher inhibitory effect (Figure 2 and Table 2).

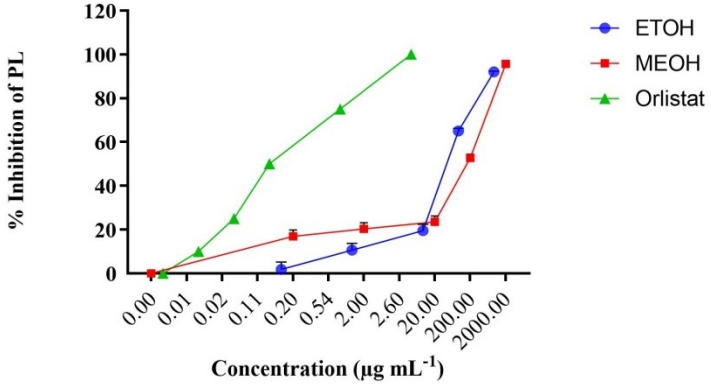

Figure 2. *In vitro* pancreatic lipase (PL) inhibition by *Rubus ulmifolius* ethanolic and methanolic extracts and orlistat. Data are presented as mean ± SD (n=3).

Table 2. *In vitro* IC_{50} values (μg mL^{-1}) for pancreatic lipase (PL) inhibition by *Rubus ulmifolius* extracts and orlistat.

Tested extract/compound	IC_{50} (μg mL^{-1})
Rubus ethanol extract	30.2±1.1[a]
Rubus methanol extract	120.2±8.8[b]
Orlistat	0.11± 0.0[c]

Data are presented as mean ± SD (n = 3 independent replicates). The IC_{50} values of each extract and orlistat were compared using one-way ANOVA followed by Tukey's post hoc multiple comparisons test. IC_{50} values with different letters (a-c) indicate statistically significant differences ($p < 0.001$).

The *in vitro* pancreatic lipase inhibition assay showed that, orlistat was the most potent inhibitor (IC_{50}= 0.11 μg mL^{-1}), as expected given its clinical use as an anti-obesity drug. However, the results also indicate that both *Rubus ulmifolius* extracts possess inhibitory activity; the ethanolic extract was significantly more potent (IC_{50}= 30.2 μg mL^{-1}) compared to the methanolic extract (IC_{50}= 120.2 μg mL^{-1}). This difference in activity suggests that the active compounds are preferentially extracted by ethanol, demonstrating the critical role of solvent selection in obtaining potent extracts.

HPLC results

HPLC-DAD-UV analysis (Tables 3 and 4) revealed that the flavonoid rutin was the most abundant component in both the ethanolic and methanolic extracts of *R. ulmifolius*, with concentrations ranging from 6.173 to 7.092 mg 100 mg^{-1}.

Table 3. Identified compounds and their concentrations in *Rubus ulmifolius* ethanolic extract.

Compound name	Retention time (min.)	Concentration in final solution (μg mL^{-1})	Normalized amount (mg 100 mg^{-1})
Caffeic acid	119.551	3.76	0.376
Kaempferol	119.162	0.44	0.044
Chlorogenic acid	118.77	3.03	0.303
Coumaric acid	70.6415	3.96	0.396
Epicatechin hydrate	119.137	8.94	0.894
Gallic acid	ND	ND	ND
Quercetin	112.865	0.042	0.0042
Riboflavin	119.553	6.96	0.696
Rutin	86.473	61.73	6.173
Tannic acid	119.139	17.92	1.792
Vanillic acid	42.539	7.00	0.700
Vanillin	64.58	3.02	0.302

Table 4. Identified compounds and their concentrations in *Rubus ulmifolius* methanolic extract.

Compound Name	Retention time (min.)	Concentration in final solution (μg mL^{-1})	Normalized amount (mg 100 mg^{-1})
Caffeic acid	119.303	2.98	0.298
Kaempferol	119.241	0.38	0.038
Chlorogenic acid	118.89	7.06	0.706
Coumaric acid	70.572	5.00	0.500
Epicatechin hydrate	118.877	6.07	0.607
Gallic acid	ND	ND	ND
Quercetin	112.25	4.59	0.459
Riboflavin	ND	ND	ND
Rutin	86.438	70.92	7.092
Tannic acid	119.22	18.14	0.1814
Vanillic acid	42.61	3.72	0.372
Vanillin	65.41	8.53	0.853

While rutin was predominant in both, levels of the second major component, tannic acid differed, with the ethanolic extract at 0.1792 mg 100 mg^{-1} and the methanolic extract at 0.1814 mg 100 mg^{-1}. These differences highlight the effect of solvent polarity on the composition of the extracts. Representative HPLC chromatograms at UV wavelengths of 265, 330, and 370 nm are provided in Figures 3 and 4.

GC-MS results

The chemical compositions of ethanolic and methanolic extracts from *Rubus ulmifolius* were analyzed using gas chromatography-mass spectrometry (GC-MS). The total ion chromatograms (TIC), which represent the compound profiles, are presented in Figures 5 and 6, with their corresponding compound identifications and relative percentages listed in Tables 5 and 6, respectively.

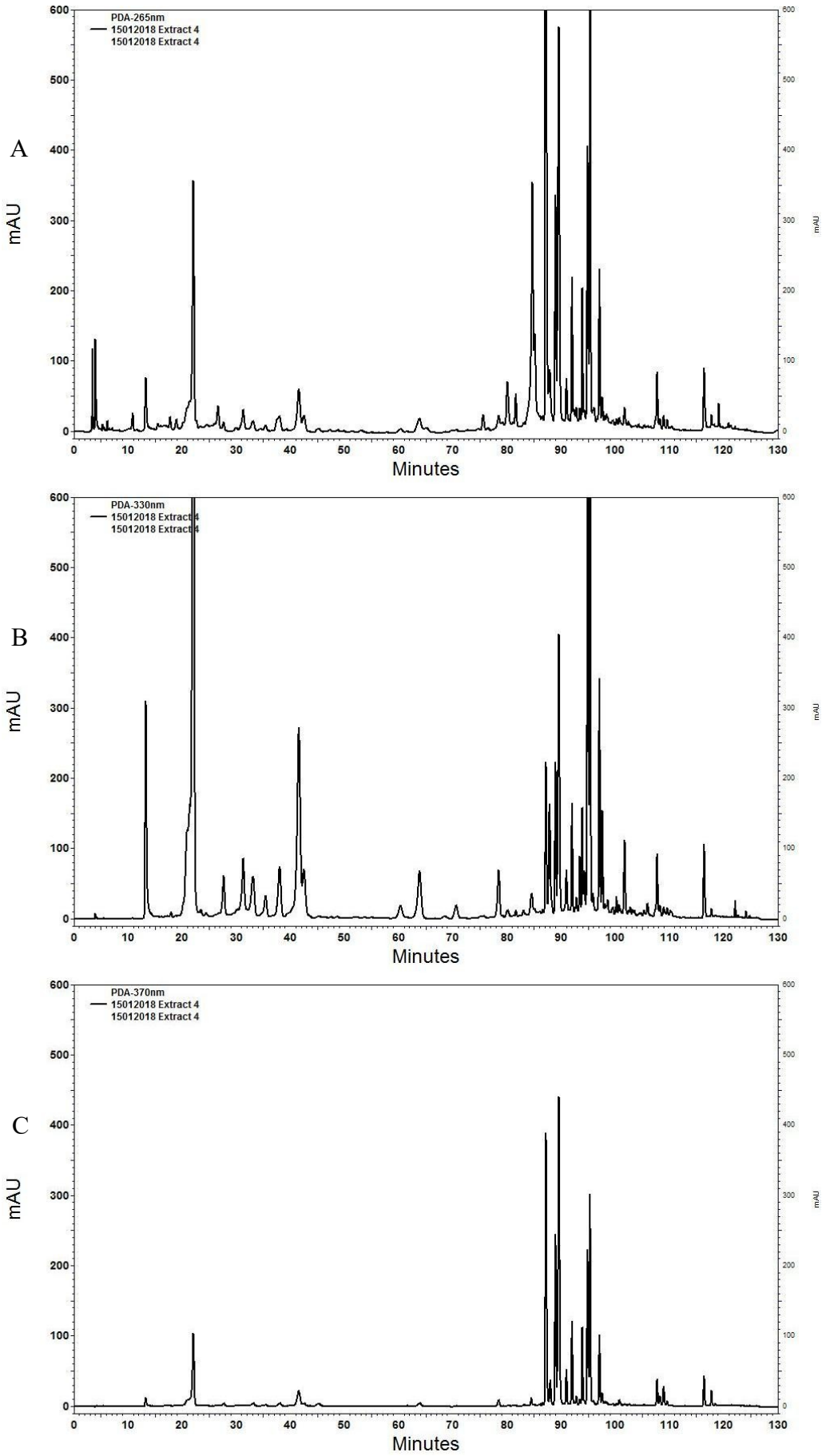

Figure 3. HPLC chromatogram of *Rubus ulmifolius* ethanolic extract. (A) Detected at 370 nm, (B) Detected at 330 nm, (C) Detected at 265 nm.

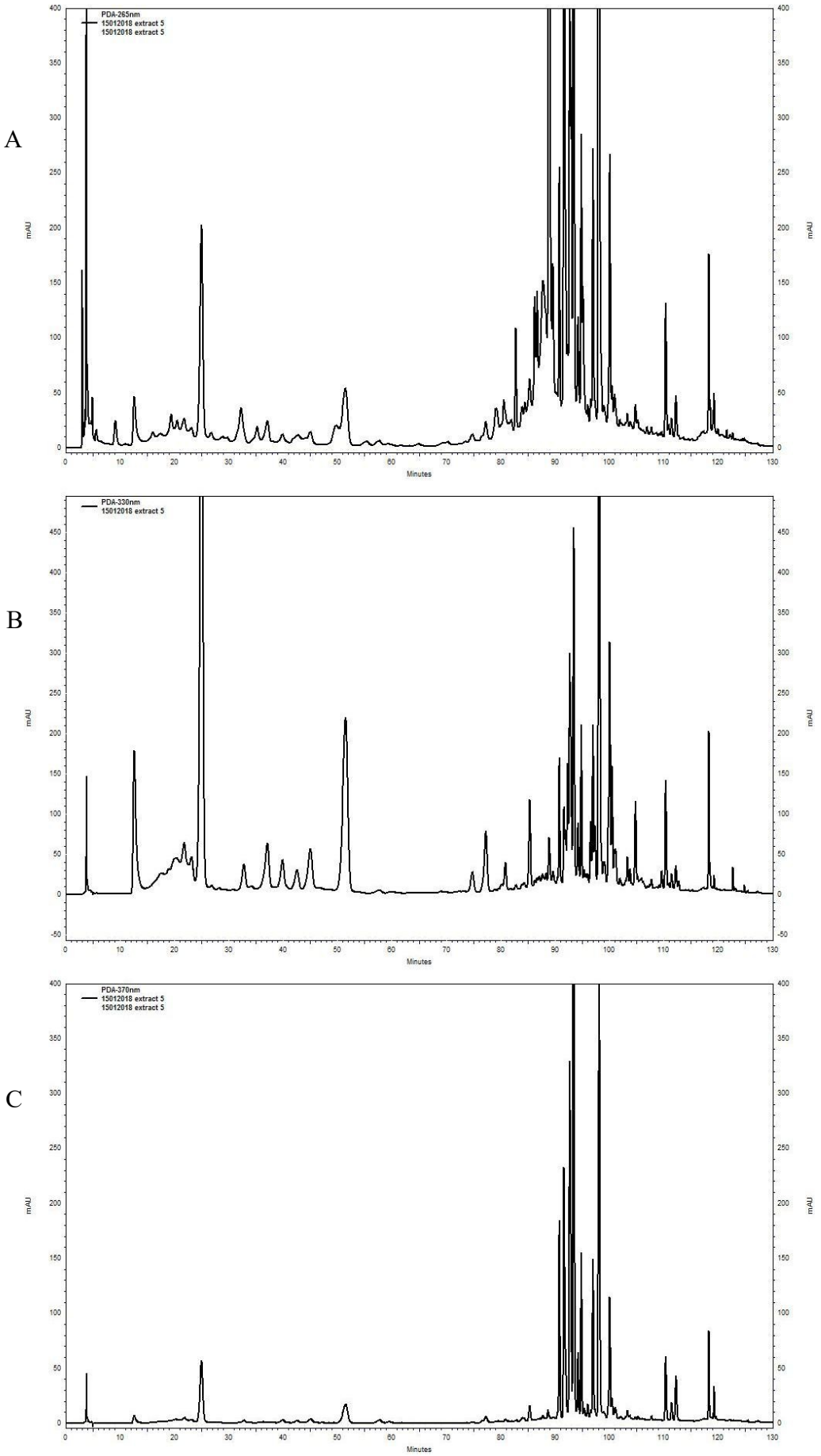

Figure 4. HPLC chromatogram of *Rubus ulmifolius* methanolic extract. (A) Detected at 370 nm, (B) Detected at 330 nm, (C) Detected at 265 nm.

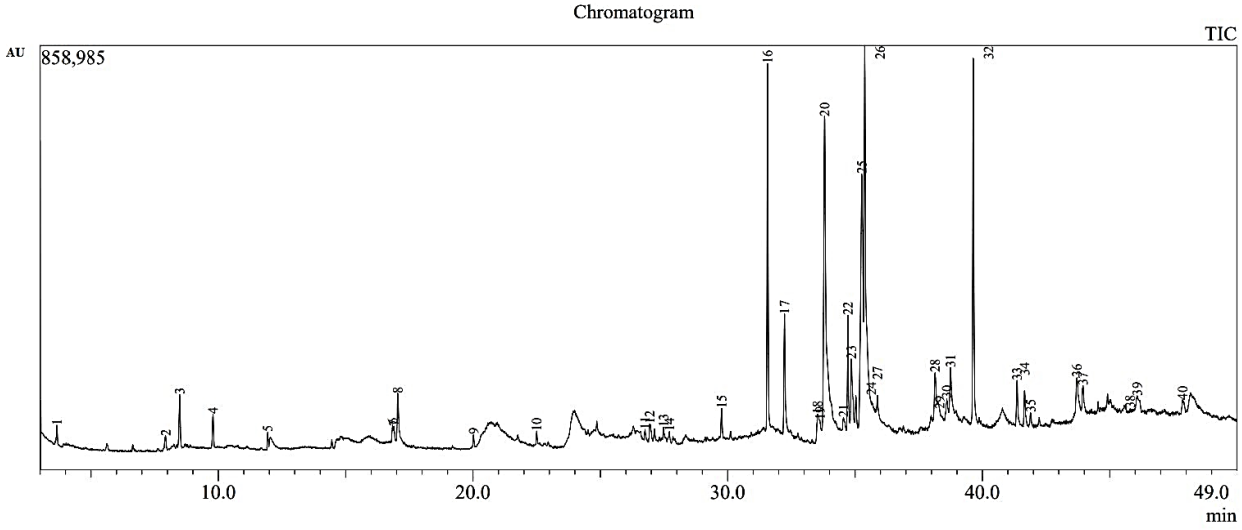

Figure 5. Total Ion Chromatogram (TIC) of *Rubus ulmifolius* ethanolic extract.

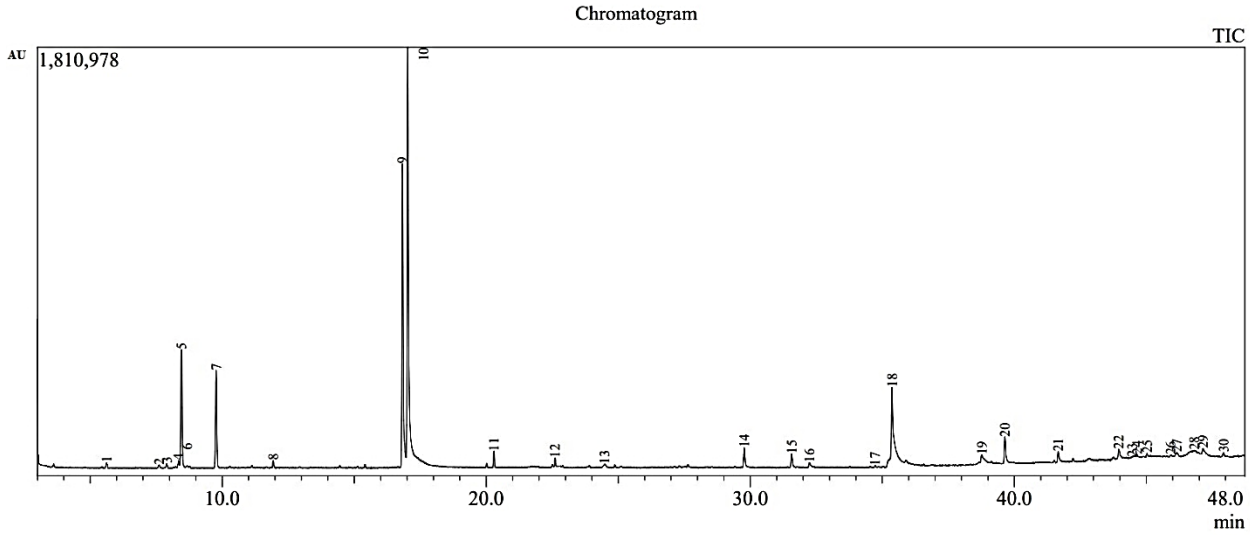

Figure6. Total Ion Chromatogram (TIC) of *Rubus ulmifolius* methanolic extract.

Table 5. Phytocomponents identified by GC-MS in *Rubus ulmifolius* ethanolic extract.

Peak	Constituents	RT	Area (%)	m/z
1	Methane, diethoxy-	3.662	0.45	59.00
2	α-Phellandrene	7.927	0.40	93.05
3	O-Cymene	8.482	1.45	119.05
4	γ-Terpinene	9.791	0.83	93.05
5	Camphor, (1R,4R)-(+)-	11.938	0.34	95.05
6	Thymol	16.840	0.45	135.05
7	2-Dodecanone	16.895	0.34	58.00
8	Carvacrol	17.044	1.47	135.00
9	Dichloroacetic acid, nonyl ester	20.019	0.29	69.05
10	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	22.500	0.25	191.05
11	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	26.756	0.26	135.05
13	Undecanoic acid, 11-bromo-, methyl ester	27.480	0.32	74.05
14	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	27.707	0.39	135.05
15	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	29.757	0.73	149.00
16	Hexadecanoic acid, methyl ester	31.565	9.23	74.00
17	I-(+)-Ascorbic acid 2,6-dihexadecanoate	32.230	3.58	73.00
20	Oleic Acid	33.804	17.62	55.05
21	9,12-Octadecadienoic acid (Z,Z)- eic Acid	34.548	0.55	41.05
22	9-Octadecenoic acid (Z)-, methyl ester	34.719	2.85	55.05
23	8-Octadecenoic acid, methyl ester, (E)-	34.855	3.04	55.05
24	Phytol	35.031	0.90	71.05

25	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	35.264	12.81	79.05
26	6-Octadecenoic acid (Z)-	35.380	15.36	55.05
27	Octadecanoic acid	35.876	0.46	73.00
28	22- Tricosenoic acid	38.140	1.97	55.05
32	Hexanedioic acid, bis(2-ethylhexyl) ester	39.641	9.61	129.05
33	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	41.361	1.55	149.00
34	1,2-Benzenedicarboxylic acid, diisooctyl ester	41.655	1.27	149.00
35	1-[3-(2,6,6-Trmethyl-cyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone	41.890	0.44	135.10
36	4,8,13-Cyclotetradecatriene- 1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	43.709	1.82	81.05
37	4-(2,2,6-Trimethyl-bicyclo[4.1.0] hept-1-yl)-butan-2-one	43.948	1.02	81.00
40	Triacontane, 1-bromo-	47.857	0.69	57.05

Table 6. Phytocomponents identified by GC-MS in *Rubus ulmifolius* methanolic extract.

Peak	Constituents	RT	Area (%)	m/z
1	Origanene	5.620	0.35	93.05
2	Ethanone, 1-cyclopropyl-2-(4-pyridinyl)-	7.607	0.28	41.00
3	α–phellandrene	7.901	0.40	93.05
4	Isoterpinolene	8.343	0.45	121.05
5	O-Cymene	8.454	8.74	119.05
6	1,4-Methano-1H-Cyclopropa[d]pyridazine, 4,4a,5,5a-tetrahydro-6,6-dimethyl-, (1.alpha., 4.alpha., 4a.alpha., 5a.alpha.)-	8.670	0.23	93.05
7	γ–Terpinene	9.770	6.46	93.05
8	Camphor, (1R,4R)-(+)-	11.928	0.46	95.05
9	Thymol	16.822	21.14	135.05
10	Carvacrol	17.029	35.87	135.05
11	Caryophyllene	20.289	0.84	93.05
12	β–Bisabollene	22.606	0.44	93.05
13	Butanoic acid, anhydride	24.482	0.39	71.05
14	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	29.766	1.37	149.00
15	1,2-Benzenedicarboxylic acid, butyl octyl ester	31.570	1.09	149.00
18	Oleic Acid	35.372	11.41	55.05
19	1,2-Oxathiane, 6-dodecyl-, 2,2-dioxide	38.764	1.51	55.05
20	Hexanedioic acid, bis(2-ethylhexyl) ester	39.645	2.13	129.05
21	1,2-Benzenedicarboxylic acid, diisooctyl ester	41.658	1.05	149.00
22	1,4-Methanoazulen-7(1H)-one, octahydro-4,8,8,9-tetramethyl-, (+)-	43.952	0.95	81.05
25	Phytol	45.026	0.23	98.10
27	Sulfurous acid, octadecyl 2-propyl ester	46.150	0.51	57.05
30	Heptadecane	47.929	0.32	57.05

The GC-MS analysis showed that the two extracts differed significantly in their composition. The ethanolic extract was rich in fatty acids and their derivatives, including oleic acid (17.62%), (Z)-6-octadecenoic acid (15.36%), methyl (Z,Z,Z)-9,12,15-octadecatrienoate (12.81%), bis(2-ethylhexyl) hexanedioate (9.61%), and methyl hexadecanoate (9.23%), alongside lesser amounts of -(+)-ascorbic acid 2,6-dihexadecanoate (3.58%), carvacrol (1.47%), and o-cymene (1.45%). The methanolic extract, in contrast, was characterized by higher relative abundances of monoterpenoids, such as carvacrol (35.87%), thymol (21.14%), and o-cymene (8.74%), as well as significant amounts of oleic acid (11.41%) and γ-terpinene (6.46%).

Discussion

The National Cancer Institute considers crude extracts with antiproliferative IC_{50} values below 30 μg mL^{-1} promising candidates for further anticancer investigation (Cherbal et al., 2022). In this context, the methanolic extract of *Rubus ulmifolius* showed notable activity against the CACO2 colorectal cancer cell line, meeting the spirit of this benchmark. Across our colorectal panel over 72 hours, cisplatin displayed lower IC_{50} values than all plant extracts, as expected for a standard chemotherapeutic. However, unlike cisplatin, *R. ulmifolius* extracts were markedly less cytotoxic to normal fibroblasts, indicating preliminary tumor selectivity. Bioactivity also depended on solvent: the methanolic extract was more active in CACO2 and SW480, whereas the ethanolic extract favored CACO2 and HT29, underscoring the influence of solvent polarity on the extracted metabolome.

These observations parallel work on *Ephedra alata* from Jijel, Algeria, where an ethanolic extract inhibited proliferation and induced apoptosis in several cancer cell lines, including CACO2, with evidence for mitochondrial involvement via redox modulation and BAX upregulation (Bensam et al., 2023). Together, these findings support further interrogation of apoptosis-related mechanisms in *R. ulmifolius*.

A plausible mechanistic axis involves oxidative stress. Reactive oxygen species promote DNA damage, mutagenesis, and oncogenic transformation; conversely, phenolic-rich matrices can modulate these processes (Olas, 2018; Oszmiański et al., 2011). Our HPLC-DAD profiling detected caffeic, chlorogenic, coumaric, tannic, and vanillic acids; kaempferol, epicatechin hydrate, quercetin, and rutin; plus vanillin in both extracts and riboflavin uniquely in the ethanolic extract. These constituents are characteristic of *Rubus* spp. and align with reports that berry polyphenols inhibit multiple human cancer cell lines in a dose-dependent fashion, with cell-type-specific sensitivities (Dai & Mumper, 2010; Schulz et al., 2019; Sisti et al., 2008; Zhang et al., 2013).

Rutin was abundant in both extracts, consistent with literature describing its chemopreventive potential and antioxidant activity (Farha et al., 2020). GC–MS further differentiated the solvent fractions: the ethanolic extract was enriched in fatty acids and derivatives—oleic acid (17.62%), 6-octadecenoic acid (Z) (15.36%),9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z) (12.81%), hexanedioic acid bis(2-ethylhexyl) ester (9.61%), and hexadecanoic acid methyl ester (9.23%)—whereas the methanolic extract contained higher levels of monoterpenoids—carvacrol (35.87%), thymol (21.14%), o-cymene (8.74%), and γ-terpinene (6.46%)—alongside oleic acid (11.41%). The prevalence of saturated and unsaturated fatty acids agrees with patterns reported for congeners in the genus (Schulz et al., 2019).

Several of these components have independent anticancer precedents. Oleic acid is associated with antiproliferative and anti-invasive effects across tumor models (Deng et al., 2023; Kimura, 2002; Martínez et al., 2005), and oleic-acid–rich almond oil inhibited colon carcinoma cells (Mericli et al., 2017). Carvacrol and thymol contribute additional bioactivity: essential-oil studies document antimicrobial, anti-mutagenic, anti-inflammatory, antioxidant, and antitumor properties (Belhattab et al., 2005; Gunes-Bayir et al., 2022; Mączka et al., 2023), with thymol specifically showing anticancer effects in gastric models (Kang et al., 2016; Sobczak et al., 2014). Taken together, these data argue for a multi-component basis of activity rather than a single dominant driver.

Beyond antiproliferative effects, both extracts inhibited pancreatic lipase *in vitro*, with stronger inhibition by the ethanolic extract. This functional difference plausibly reflects compositional biases: fatty-acid-rich ethanolic fractions may favor lipase inhibition, whereas thymol/carvacrol-rich methanolic fractions can, in some settings, enhance digestive enzyme activities (Hashemipour et al., 2013). Given the epidemiologic and mechanistic links among lipase activity, overweight/obesity, and cancer (Mopuri&Meriga, 2014), the antilipase activity of *R. ulmifolius* is noteworthy. It is also coherent with prior demonstrations that phenolic-rich extracts can inhibit lipases (Ado et al., 2013; He et al., 2023) and that complex matrices—such as peanut shell extracts rich in luteolin, caffeic and ferulic acids, benzoic acid, and fatty acids—exert multi-lipase inhibition (Zheng et al., 2010). Reported lipase inhibition by diverse culinary plants (Daniş et al., 2015) further supports this pharmacological niche. Polyphenol–polyphenol interactions may shape both potency and stability, suggesting that synergy should be explicitly evaluated in future work (Hashemipour et al., 2013).

Overall, our data position *R. ulmifolius* leaf extracts as solvent-defined mixtures whose composition tracks with function: methanolic fractions, enriched in monoterpenoids (carvacrol, thymol), showed stronger *in vitro* antiproliferative activity in CACO2, while ethanolic fractions, enriched in fatty acids, produced greater antilipase effects. The combination of preliminary tumor selectivity, a phytochemical profile consistent with known anticancer polyphenols and fatty acids, and ancillary metabolic enzyme inhibition is encouraging. In line with NCI's pragmatic IC_{50} threshold for crude extracts (Cherbal et al., 2022) and analogies to apoptosis observed in other botanicals (Bensam et al., 2023), the next steps should include bioassay-guided fractionation to localize active principles; orthogonal confirmation of apoptosis and oxidative-stress pathways; and counter-screening in normal cells to refine selectivity. Such mechanistic and isolation studies will clarify whether individual constituents, defined sub-fractions, or synergistic constellations best account for the observed activities and will determine the translational potential of *R. ulmifolius* as a source of candidate therapeutics.

Conclusion

Rubus ulmifolius leaf extracts demonstrate solvent-dependent bioactivity, with the methanolic extract showing greater antiproliferative effects against CACO2 cells and the ethanolic extract exhibiting stronger

antilipase activity. This variation is attributed to their differing phytochemical profiles. The methanolic extract is rich in monoterpenoids (e.g., carvacrol, thymol), while the ethanolic extract is enriched with fatty acids. Both extracts contain various polyphenols, with rutin being a key shared constituent. These findings underscore the importance of solvent choice in drug discovery and warrant further investigation to identify the specific active compounds and their modes of action.

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