https://doi.org/10.31925/farmacia.2026.3.14 **ORIGINAL ARTICLE**

IMPACT OF SYRINGIC AND CAFFEIC ACIDS ON EPITHELIAL-MESENCHYMAL TRANSITION MARKERS IN COLORECTAL CANCER CELL LINES

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Abstract

One of the leading causes of mortality among malignancies is colorectal carcinoma (CRC). As metastasis is central to CRC progression, research on the anti-invasive and anti-migratory actions of bioactive compounds is crucial for advancing new treatments. Biologically active plant compounds, including phenolic acids, are known for their therapeutic properties. Both syringic (SA) and caffeic acids (CA) are recognized for their significant antioxidant, anti-inflammatory, and anticancer activity. In this study, the influence of these acids on the anticancer, invasive, and migratory potential of CRC cells was examined, including the expression of epithelial-mesenchymal transition (EMT) and invasion markers. For these purposes, the MTT assay, wound healing assay, transwell method, real-time PCR, and immunocytochemistry were used. Results showed low cytotoxic effects, but significant anti-invasive and anti-migratory effects. Expression analyses indicated modulation of key monitored EMT and invasion markers on transcriptional and protein levels, suggesting a partial EMT transition characterized by incomplete induction of invasive properties. All results indicate that SA and CA exhibit potential anti-invasive and anti-migratory properties in the tested cells, independently of non-cytotoxic effects, opening a new field of research.

Rezumat

Carcinomul colorectal (CRC) reprezintă una dintre principalele cauze de mortalitate asociate afecțiunilor maligne. Compușii bioactivi de origine vegetală, precum acizii fenolici, sunt recunoscuți pentru proprietățile lor terapeutice. Atât acidul siringic (SA), cât și acidul cafeic (CA) sunt cunoscuți pentru activitatea antioxidantă, antiinflamatoare și anticancerigenă. În cadrul acestui studiu, a fost evaluată influența acestor acizi asupra potențialului anticancerigen, invaziv și migrator al celulelor de CRC, precum și asupra expresiei markerilor implicați în tranziția epitelio-mezenchimală (EMT) și în invazie. În acest scop, au fost utilizate testul MTT, testul de vindecare a plăgii, metoda *transwell*, reacția de polimerizare în lanț în timp real și imunocitochimia. Rezultatele au evidențiat efecte citotoxice reduse, însă efecte antiinvazive și antimigratorii semnificative. De asemenea, a fost observată o modulare a principalilor markeri monitorizați ai EMT și ai invaziei, atât la nivel transcripțional, cât și la nivel proteic, sugerând o tranziție EMT parțială, caracterizată prin inducerea incompletă a proprietăților invazive. În concluzie, rezultatele indică faptul că SA și CA prezintă un potențial antiinvaziv și antimigrator în celulele testate, independent de efectele citotoxice.

Keywords: migration, invasion, CRC

Introduction

According to the International Agency for Research on Cancer (1), colorectal cancer (CRC) is the 3rd most common cancer worldwide. The most frequently diagnosed type of CRC is adenocarcinoma, which arises from the malignant transformation of colon and rectal cells, with the potential to invade surrounding tissues and form distant metastatic lesions (2). The treatment options for CRC become more limited with the appearance of metastases. During disease progression, CRC cells develop resistance and undergo morphological changes, mainly driven by the epithelial-mesenchymal transition (EMT). Through this process, the cells acquire mesenchymal cell characteristics, such as weaker adhesion and intercellular communication, resulting in the development of strong invasive and migratory abilities (3).

At the molecular level, EMT-driven changes in cell phenotype and function are controlled by complex transcriptional programs, with nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) as a key regulator of invasion and migration. Namely, NFκB is a transcription factor that regulates the expression of genes involved in invasion and migration, among others. It induces the expression of urokinase (uPA) and metalloproteinase 9 (MMP-9), which degrade components of the extracellular matrix and basement membrane, enabling cell migration (4, 5). Molecular markers of invasion and

migration, such as β-catenin, SNAIL-1, vimentin, and intercellular adhesion molecule-1 (ICAM-1), are regulated by NFκB (6, 7). They promote EMT by reducing epithelial characteristics and causing migration by binding cells with migratory potential to the wall of blood vessels (8, 9). Given its ability to simultaneously regulate proteolytic activity, cell adhesion, and EMT-associated transcriptional programs, NFκB represents a critical target for modulating invasive and migratory behavior in colorectal cancer cells.

In this context, naturally occurring polyphenolic compounds have gained increasing attention as potential modulators of NFκB-driven tumor progression. Syringic (SA) and caffeic (CA) acids belong to the group of polyphenolic compounds, more precisely phenolic acids. They are present in plants and fungi, including fruits, vegetables, nuts, and cereals. These acids have numerous medical benefits and have been shown to have antioxidant, anticancer, antimicrobial, and anti-inflammatory effects (10, 11, 12). It is particularly significant that in CRC cell lines, it has been shown that SA can induce apoptosis, inhibit proliferation, reduce NFκB activity and proteasome regulation, indicating its potential to inhibit cell invasion and migration (13). Caffeic acid, on the other hand, can inhibit key signaling pathways involved in tumor progression, including NFκB, STAT3, ERK1/2, and AP-1, resulting in decreased expression of angiogenesis factor (VEGF) and invasive enzymes such as MMP-2 and MMP-9 (12).

Besides well-documented antioxidant and anti-inflammatory properties of the SA and CA, their role in cancer progression remains poorly understood, particularly regarding their influence on the invasion and migration of CRC cells, processes that are central to tumor metastasis and poor clinical outcomes. Therefore, this study aims to investigate the effects of these phenolic acids on the invasive and migratory behavior of colorectal cancer cells, with particular focus on their role as non-cytotoxic modulators of cancer progression.

Materials and Methods

Cell cultivation

Human epidermal keratinocytes (HaCaT, CLS – Cell Lines Service, Eppelheim, Germany) and human CRC cell lines (HCT-116, ATCC CCL-247, and SW-480, ATCC CCL-228, ATCC - American Type Culture Collection, Manassas, Virginia, USA) were used in this study. They were cultured in high-glucose DMEM (EuroClone, Italy), supplemented with 10% fetal bovine serum and antibiotics (Penicillin/streptomycin Solution 100x, Capricorn Scientific, Germany). Cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were grown in 75 cm^2 culture bottles containing 10 mL of complete medium, and after one passage, cells were transferred to appropriate culture vessels for the experiment. All experiments were performed with cells at 70-80% confluence, unless otherwise noted. Cell lines were used at low passage numbers. STR profiling for cell line authentication and mycoplasma testing was not performed in this study.

For all experiments, cells were treated with SA and CA, dissolved in DMEM containing 1% DMSO at 1000 µg/mL, and subsequently diluted to the target concentrations. Both compounds were purchased from Sigma-Aldrich (USA), with HPLC purities ≥95% for SA (Cat. No: S6881-5G) and ≥ 98% for CA (Cat. No: C0625-2G).

Cell cytotoxicity assay (MTT assay)

The colorimetric cell cytotoxicity assay used in this study is the MTT assay (14). For this test, HaCaT, HCT-116 and SW-480 cells were grown in a 96-well plate (10^4 cells *per* well) and treated after 24 h with 100 µL of each concentration of SA (1, 10, 50, 100, 200, 300, 400, and 500 µg/mL), and CA (1, 10, 50, 100, 200, and 300 µg/mL) for 24, 48, and 72 h. SA and CA were obtained from Sigma Aldrich (Germany). After treatment, the cells were incubated with 1 mg/mL MTT for 4 h. DMSO was used to dissolve the formazan, and the absorbance was measured at 492 nm on an ELISA microplate reader (PKL PCC 142). The percentage of viable cells was calculated as the ratio of the treated group's absorbance to the control group's absorbance, multiplied by 100. Dose–response curves were used to assess cell viability, and IC_{50} values were calculated for each treatment using appropriate software (CalcuSyn, Biosoft, Cambridge, UK). The selectivity index (SI) was calculated as the ratio of the IC_{50} values in HaCaT normal cells to those in CRC cells (15).

The concentrations of 10 and 50 µg/mL were selected for other methods because they were non-cytotoxic and allowed evaluation of their effects on invasion and migration through signaling pathways.

Cell Invasion

The invasion potential of the HCT-116 and SW-480 cells was monitored using a transwell invasion assay (16). Cells were seeded in medium without FBS (10^5 cells *per* insert) in transwell inserts (pore size 8 µm) coated with Matrigel (3 mg/mL in serum-free DMEM), with treatment at targeted concentrations or medium for control. Medium with 20% FBS was added to the well below the insert, which served as an attractant. After 24 h incubation, cells were stained with 0.1% crystal violet (Sigma-Aldrich, USA). Stained cells were destained with 10% acetic acid, and the color was measured on an ELISA microplate reader (RT-21000C) at 595 nm. The obtained absorbance was expressed as optical density, and the results were presented as a percentage of invaded cells relative to the control (100%).

Cell Migration

Transwell assay

The cell migration assay used to monitor single-cell migration in this research is the transwell assay (17). Cells (1×10^5 *per* insert) were seeded in serum-free medium with treatments or in control medium without treatment in transwell inserts (8 μm pores), with 20% FBS in the lower chamber as a chemoattractant. After 24 h, cells were stained with 0.1% crystal violet (Sigma-Aldrich, USA), destained with 10% acetic acid, and absorbance was measured at 595 nm using an ELISA microplate reader (RT-21000C). Results were expressed as optical density and presented as a percentage relative to the control (100%).

Wound healing assay

To monitor the collective migratory potential of HCT-116 and SW-480 cells, a wound-healing assay was performed (16). Cells were seeded into 6-well plates at equal volumes and cultured to 90% confluency. After reaching confluency, a scratch was made in the middle of the well with a sterile 200 μL pipette tip. Medium was added to the control cells, and the target treatment concentrations (10 and 50 μg/mL) were added to the others. The zero hour of the treatment was used as a control, and the treatment effect was monitored and visualized under a fluorescent microscope (Nikon Ti-Eclipse) at 100x magnification at 6, 12, and 24 h. Images were quantified in ImageJ. The results are expressed as a percentage of the relative wound gap and were calculated by dividing the relative wound area in the treated group by that in the control group at the corresponding time point, then multiplying by 100.

RNA isolation and Reverse transcription (RT-PCR)

HCT-116 and SW-480 cell lines were treated with targeted concentrations of the tested substances (10 and 50 μg/mL) or with fresh medium (control) and cultivated for 24 h. After the incubation period, RNA was isolated using the TRIzol method (18) with RNA extraction (Eurx, Poland). The total RNA concentration was measured on a Spectrophotometer (Eppendorf BioPhotometer) and translated into complementary DNA (cDNA) using the NG dART RT kit (EURx, Poland). Each reverse transcription reaction for mRNA, in a total volume of 40 μL, contained all components according to the kit instructions and 1 μg of RNA template. Random hexamer primers supplied with the kit were used for cDNA synthesis. The reaction mixture for reverse transcription was incubated for 10 min at 25 ºC, 50 min at 50 ºC, and then inactivated at 85 ºC for 5 min.

Relative gene expression (qRT-PCR assay)

The relative mRNA expression of target genes (NFκB, CTNNB1 (gene that codes β-Catenin), Vimentin, SNAIL1, and MMP9) was determined relative to a housekeeping gene (β-actin) from cDNA. SG/ROX qPCR Master Mix (2x) (EURx, Poland) was used in this study for gene expression analysis, with the 7500 Real-Time PCR System (Applied Biosystems 7500/7500 Fast Real-Time PCR Software ver. 2.0). Primer (Metabion, Germany) sequences used in this study are shown in Table I. Each PCR reaction, with a total volume of 20 μL, consists of the kit components at the concentrations indicated in the instructions. PCR reaction was incubated 2 min at 50 ºC and 10 min at 95 ºC, following 45 cycles of amplification (15 s on 94 ºC, 30 s on 55-60 ºC – annealing temperatures were presented in Table I, and 30 s on 72 ºC, according to the manufacturer). Melt curve analysis was performed between 55 ºC and 94 ºC. The results were calculated using a $2^{-\Delta\Delta Ct}$ method for relative gene expression (19). This method involves comparing the expression of target genes with that of an endogenous control (housekeeping gene) in both the treatment and control samples.

Table I

Primers used in the qRT-PCR reaction

Primer	Forward sequence	Reverse sequence	Annealing temperature
β-actin	5′-AAGCAGGAGTATGACGAGTCCG-3′	5′-GCCTTCATACATCTCAAGTTGG-3′	60°C
NFκB	5′-ATGGCTTCTATGAGGCTGAG-3′	5′-GTTGTTGTTGGTCTGGATGC-3′	57°C
CTNNB-1	5′-AAAATGGCAGTGCGTTTAG-3′	5′-TTTGAAGGCAGTCTGTCGTA-3′	55°C
Vimentin	5′-GGCTCAGATTCAGGAACAGC-3′	5′-AGCCTCAGAGAGGTCAGCAA-3′	60°C
SNAIL-1	5′-TCAGACGAGGACAGTGGGAAAG-3′	5′-GCTTGTGGAGCAGGGACATTC-3′	60°C
MMP-9	5′-TGCCCGGACCAAGGATACAG-3′	5′-TCAGGGCGAGGACCATAGAG-3′	57°C

Immunocytochemistry

The immunocytochemical method was used to measure protein expression of ICAM-1 (ELK Biotechnology, USA; 1:200), uPA (ELK Biotechnology, USA; 1:200), and MMP-9 (R&D systems, USA; 1:200) in CRC cell lines (HCT-116 and SW-480). The method was performed according to the protocol (20). After seeding cells on coverslips, the cells were treated with selected concentrations in treatment groups and with medium in the control group. After 24 h incubation, the cells were fixed with 4% paraformaldehyde and permeabilized with absolute methanol. Non-specific binding sites were blocked with 1% bovine serum albumin, and the samples were then washed with PBS. The cells were then incubated with primary antibodies. MMP-9 was detected using Alexa-conjugated secondary antibody (Sigma Aldrich, Germany). In contrast, uPA and ICAM-1 were detected using a Cy3-conjugated secondary antibody (Sigma-Aldrich, Germany),

both at a dilution of 1:200. Immunofluorescence staining for each marker was performed on separate coverslips and not in a multiplex manner. The nuclei were stained with DAPI (Sigma-Aldrich, Germany; 1:1000).

Cells were imaged under a fluorescent microscope (Nikon Ti-Eclipse) at 1000x magnification. The results are presented as relative fluorescence *per* cell, quantified in ImageJ by measuring the cell area and subtracting background fluorescence. At least 20 cells *per* treatment/control were analyzed to calculate the mean ± SE.

Statistical analysis

The results in this study were presented as the means of three independent experiments. The data were expressed as mean ± standard error (S.E.). One-way ANOVA was used to determine statistical significance ($p < 0.05$). The statistical analysis was performed using SPSS (SPSS for Windows, V17, Chicago, IL, USA). The effect on cell viability has flowed from dose curves. The cytotoxic effect of each tested complex was expressed by IC_{50} values, which were calculated from the dose curves by a computer program (CalcuSyn, V2.1, Biosoft, Cambridge, UK). The ImageJ software (ImageJ, V1.53a, National Institutes of Health, Bethesda, Maryland, USA) was used to measure wound width and protein expression fluorescence intensity. GraphPad Prism (GraphPad Software, V8.0.2, San Diego, CA, USA) was used to present the results graphically.

Results and Discussion

In this study, the effects of SA and CA treatments on CRC cells were investigated with a focus on their invasive and migratory potential. Initially, the antiproliferative activity of both compounds was evaluated using the MTT assay to determine viability, IC_{50} values, and selectivity indices toward CRC cells. Subsequently, the impact of the treatments on cell invasion and migration was assessed through functional assays. Finally, to explore the molecular pathways affected by the treatments, the expression of invasion and migration markers was determined at the gene and protein levels.

Antiproliferative effect

The cell viability of CRC cell lines was evaluated using the MTT assay. The results were shown in Figure 1. SA had minimal impact on cell viability at lower concentrations and may even promote proliferation. An antiproliferative effect was observed only at concentrations above 200 µg/mL, with HCT-116 cells being more sensitive compared to SW-480 cells. In contrast, CA exhibited a stronger effect. At concentrations above 200 µg/mL, viability was reduced, whereas lower concentrations showed only a moderate proliferative effect.

As summarized in Table II, the antiproliferative response was independent of treatment duration. Caffeic acid generally showed greater cytotoxicity, with lower IC_{50} values than SA, whereas SW-480 cells showed notable resistance, with IC_{50} values above 500 µg/mL. To assess the toxicity of the tested acids and their therapeutic potential, particularly on cancer cells, an SI was calculated. A higher SI indicates greater sensitivity of cancer cells compared with normal cells, suggesting that the treatment preferentially targets cancer cells. Based on the results in Table II, CA has a stronger effect on both CRC cell lines than SA does. These were consistent with the literature results (21), which have reported higher IC_{50} values for SW1116 (0.95-1.15 mg/mL) and SW837 (1.13-1.2 mg/mL) in CRC cell lines treated with SA. Mihanfar (22) showed that 48 h after treatment with SW-480, SA also had a high IC50 value (1200 µM). Notably, there is a lack of published data regarding the cytotoxicity of SA on HCT-116 cells. Analyzing the CA effect in literature, similar low cytotoxic effects have been described by Secme *et al*. (23) for HCT-116 cells (IC_{50} values 826.59 µM at 24h), and by (24) for SW-480 cells (IC_{50} values were 742.8 µg/mL at 24 h and 460.7 µg/mL at 72 h). Syringic acid showed weak activity in both colorectal cancer cell lines, with SI values below 1, indicating a lack of cancer cell selectivity. In contrast, CA demonstrated a moderately active effect, as indicated by IC_{50} values greater than 1, suggesting considerable anticancer specificity (25, 26). These findings suggest that CA represents a promising compound for further investigation as a selective anticancer agent. Nevertheless, this interpretation should be considered with caution, as HaCaT cells, although widely used as a model of normal epithelial cells, are not tissue-specific. Therefore, the inclusion of normal colon epithelial cells as a more appropriate non-malignant control would provide a more accurate assessment of anticancer selectivity.

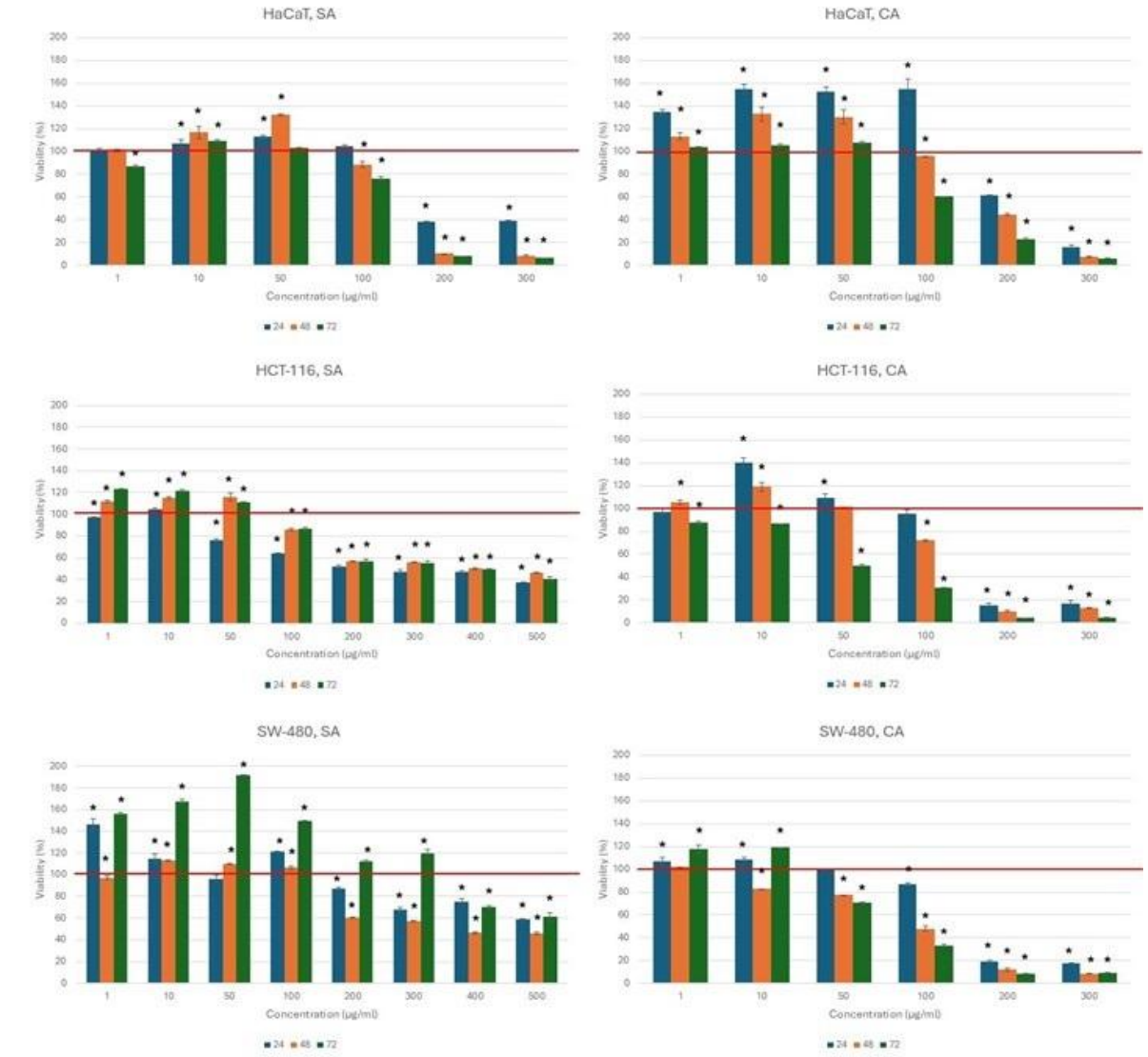

Figure 1.

The effect of SA and CA treatments on HaCaT, HCT-116, and SW-480 cell lines' viabilities, after 24, 48, and 72 h of exposure. Results are expressed as a percentage of viable cells (mean ± standard error of three independent experiments) relative to the control (100%). *indicates statistical significance ($p < 0.05$) evaluated by One-way Anova using Tukey post hoc test, treatment *vs*. control

Table II

Antiproliferative effect - IC_{50} values (µg/mL) and selectivity index - SI of SA and CA treatments on HaCaT, HCT-116, and SW-480 cell lines after 24, 48, and 72 h exposure. IC_{50} values are calculated based on percentages of viable cells, and SI results are calculated based on IC_{50} values

Treatment		IC_{50} (µg/mL)		SI	
		SA	CA	SA	CA
HaCaT	24 h	172.92 ± 0.08	222.98 ± 3.14	ND	ND
	48 h	144.66 ± 1.71	180.93 ± 0.65	ND	ND
	72 h	117.21 ± 0.99	132.24 ± 0.71	ND	ND
HCT-116	24 h	242.27 ± 1.85	158.72 ± 4.41	0.713 ± 0.005	1.407 ± 0.059
	48 h	400.3 ± 2.8	138.14 ± 0.85	0.361 ± 0.002	1.31 ± 0.013
	72 h	372 ± 1	50.01 ± 0.65	0.315 ± 0.003	2.645 ± 0.048
SW-480	24 h	> 500	149.25 ± 1.91	ND	1.494 ± 0.04
	48 h	367.43 ± 2.06	96.14 ± 4.27	0.394 ± 0.007	1.884 ± 0.077
	72 h	> 500	77.05 ± 1.27	ND	1.717 ± 0.038

Results are presented as the mean ± standard error of three independent experiments. ND – Non-determined

Invasive and migratory potential

The anti-invasive and anti-migratory effects of SA and CA on HCT-116 and SW-480 CRC cell lines were tested by the Transwell (with and without Matrigel) and Wound Healing assays. As shown in Figure 2, both treatments significantly reduced the percentage of invaded and migrated cells. In HCT-116 cells, SA was more effective at low doses, and CA at high doses, whereas in SW-480 cells, both acids had stronger responses at low concentrations, indicating distinct mechanisms.

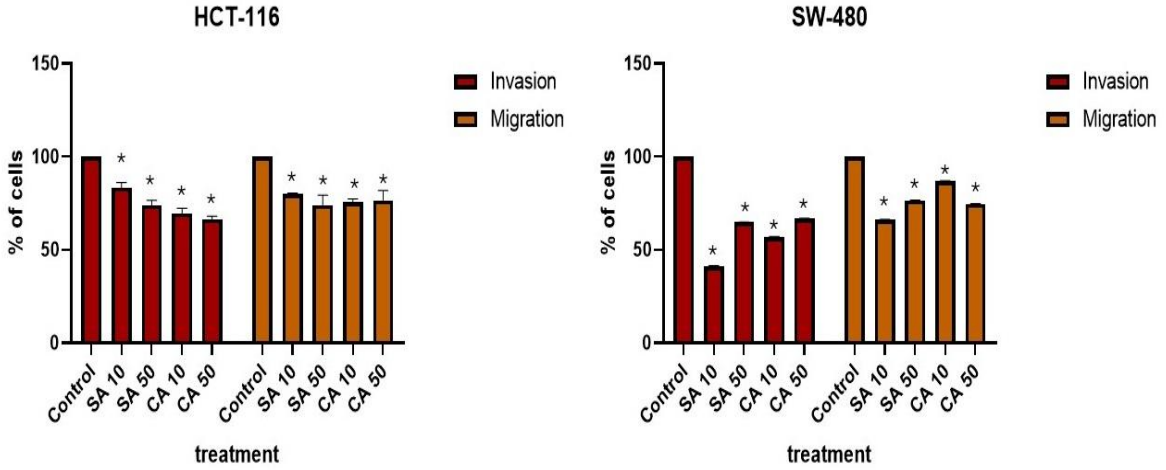

Figure 2.

Anti-invasive and anti-migratory potential of CRC cells after 24 h of incubation with targeted concentrations of SA and CA (μg/mL). Results are expressed as percentages (the mean ± standard error of three independent experiments) relative to the control (100%). *indicates statistical significance ($p < 0.05$) evaluated by One-way Anova using Tukey post hoc test, treatment *vs*. control

The Wound Healing Assay results shown in Figure 5 were calculated based on the micrographs presented in Figures 3 and 4. As shown in the micrographs (Figures 3 and 4), untreated HCT-116 control cells migrated by forming a distinct migratory front, gradually closing the wound area, or by detachment of cell clusters that migrated collectively. In contrast, SW-480 cells exhibited a predominantly single-cell migration pattern. Our results indicate that SW-480 cells exhibit higher basal migration than HCT-116 cells. Overall, collective migration observed in the Wound Healing Assay was weaker than the individual migration measured by the Transwell Assay. This difference is expected, as the two methods assess distinct aspects of migratory potential. SA at both concentrations reduced collective migration, with a pronounced inhibition at the higher concentration in SW-480 cells. In contrast, CA demonstrated a clear dose-dependent effect in HCT-116 cells, while in SW-480 cells, a stronger inhibitory effect was observed at the lower concentration. Those results suggest a cell line–specific response. Consistent with our results, (21) presented similar effects of SA on the invasive and migratory potential of SW1116 and SW837 CRC cells. Additionally, Secme *et al*. (23) reported that CA suppressed the invasive potential of HCT-116 cells, with 79.74% of cells invading. Park *et al*. (27) also described the inhibited migratory capacity of HCT-116 cells. These results suggest that both tested phenolic acids exert anti-invasive and anti-migratory effects, although their efficacy is cell line–dependent, with SW-480 cells showing greater sensitivity to treatment. This interpretation should be considered in the context of the experimental design, as the wound healing assay was performed over a 24 h period, which predominantly reflects cell migration (28). However, in the absence of a proliferation inhibitor, a contribution of cell proliferation to wound closure cannot be entirely excluded. Therefore, the observed effects likely represent a combined influence on cell motility and proliferation-related processes.

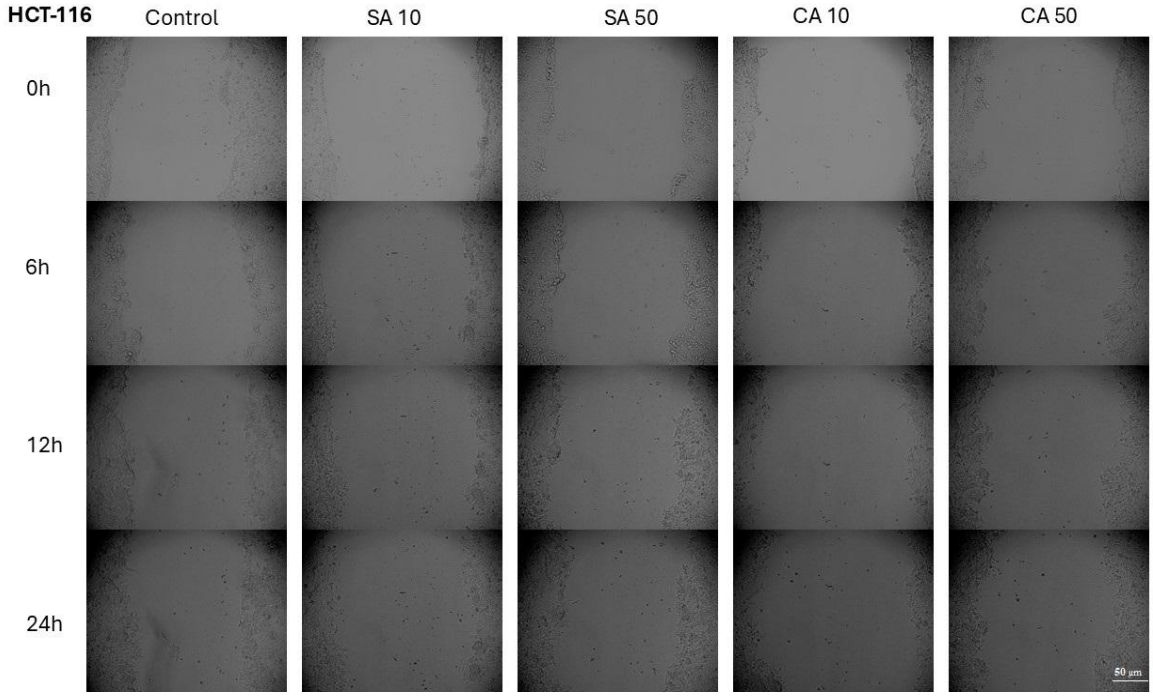

Figure 3.
Micrographs of the gap in the Wound healing assay in HCT-116 control and treated cells at different time points (0, 6, 12, and 24 h)

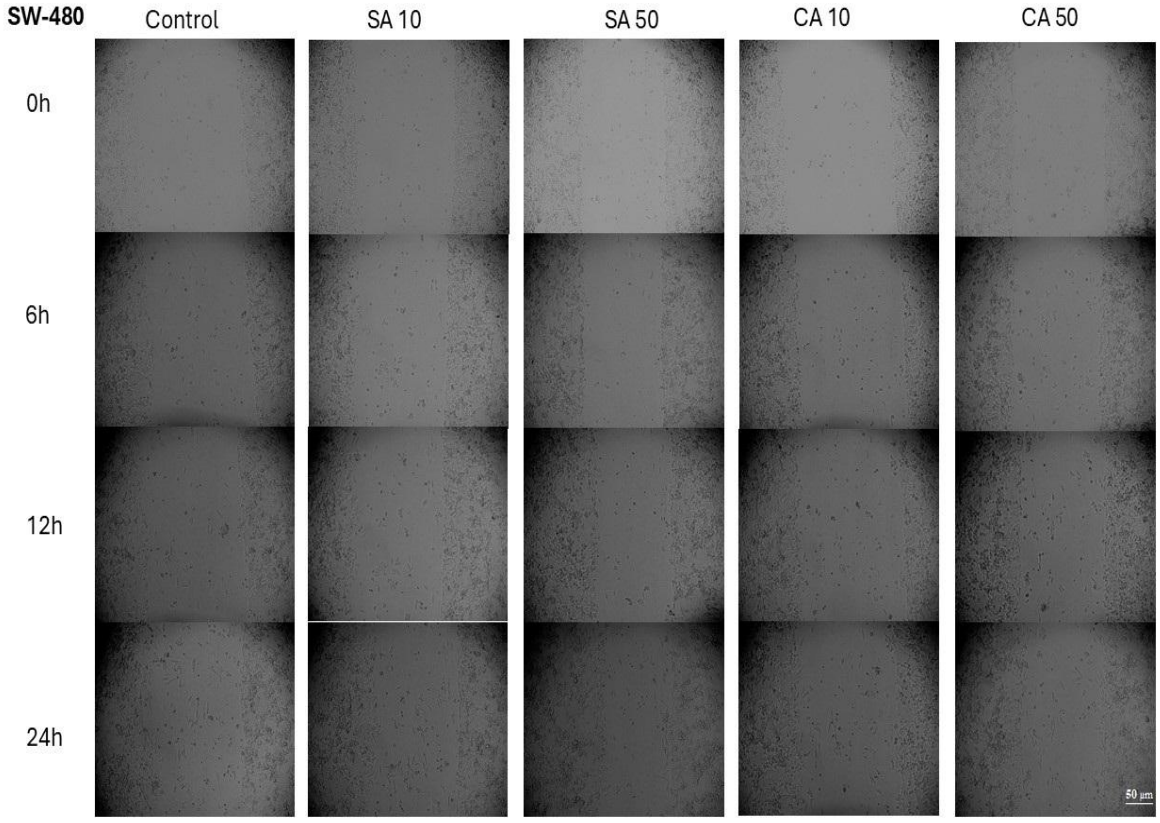

Figure 4.
Micrographs of the gap in the Wound healing assay in SW-480 control and treated cells at different time points (0, 6, 12, and 24 h)

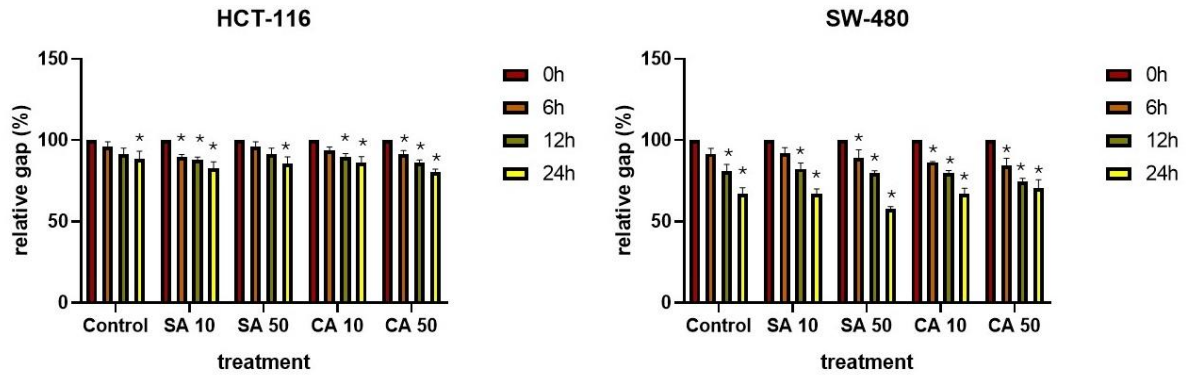

Figure 5.

Effects of SA and CA on the collective migration of CRC cells are presented as relative changes in gap width (mean ± standard error of three independent experiments) relative to the initial (zero) hour. Results are presented as the mean ± standard error of three independent experiments. *indicates statistical significance ($p < 0.05$) evaluated by One-way Anova using Tukey post hoc test, treatment *vs*. 0 h

Invasive and migratory markers on gene and protein levels

The expression of genes, including the transcription factor *NFκB*, the EMT-associated marker *CTNNB-1*, and the pro-invasive markers *VIMENTIN, SNAIL-1*, and *MMP-9,* was evaluated to assess changes in gene expression profiles potentially associated with *NFκB* signaling's role in regulating invasion and migration under SA and CA treatments. However, it should be noted that *NFκB* expression does not directly reflect NFκB transcriptional activity, which is primarily regulated post-translationally. Therefore, functional implications remain limited. In parallel, the protein levels of uPA and MMP-9, key mediators of extracellular matrix degradation, and ICAM-1, a molecule associated with cell adhesion and metastatic dissemination, were analyzed. This integrated gene and protein profiling approach enables a preliminary assessment of potential associations within the NFκB–uPA–MMP-9 regulatory axis, rather than a direct evaluation of NFκB pathway activity as a target of bioactive compounds with potential modulatory effects on tumor invasion.

Syringic acid modulated the expression of EMT-related markers in a concentration-dependent manner in HCT-116 cells. At lower concentrations, SA induced *SNAIL-1* and *CTNNB-1* expression, suggesting activation of EMT-associated pathways; however, the downregulation of Vimentin and the suppression of MMP-9 indicate that cytoskeletal reprogramming and an invasive phenotype were not achieved (Figure 6). These findings, together with the observed decrease in *NFκB* expression, need to be interpreted in light of NFκB signaling's post-translational regulation. Further functional studies would be required to clarify its role in EMT regulation, while involvement of other signaling pathways, including Wnt/β-catenin (29), PI3K/AKT (30), Hedgehog-GLI, and NOTCH (31), cannot be excluded. These were not directly investigated in this study. In contrast, higher concentrations of SA suppressed *SNAIL-1* and *CTNNB-1* expression while inducing *Vimentin* expression, which may be consistent with stress-associated cytoskeletal remo-deling rather than functional EMT. Importantly, invasion-associated mediators were downregulated at all concentrations, correlating with the observed anti-migratory and anti-invasive effects. Partial EMT involves modulation of EMT-regulating transcription factors (*SNAIL-1, CTNNB-1,* and *NF-κB*), together with partial changes in mesenchymal markers and invasion-related mediators (MMP-9, uPA, and ICAM-1) (32). In contrast, stress-associated cytoskeletal remodeling involves alterations in structural cytoskeletal components typical of mesenchymal cells. Still, it occurs in the absence of activation of EMT transcriptional regulators and invasion-associated functional proteins (33). In SW-480 cells, SA induced upregulation of *SNAIL-1* as a mesenchymal marker at both tested concentrations; however, this was followed by suppression of *CTNNB-1* and invasion-associated proteins ICAM-1, uPA, and MMP-9 (results of protein expression were calculated based on micrographs shown in Figures 7 and 8 and presented in Figure 9). Importantly, inhibition of key proteolytic mediators at the protein level suggests that SA interferes with EMT function, resulting in a less invasive phenotype despite mesenchymal marker induction.

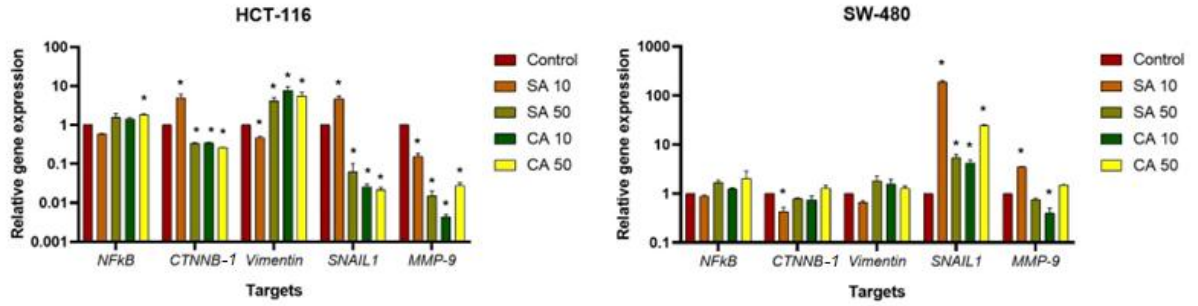

Figure 6.
Relative gene expression of target genes (NFκB, CTNNB-1, Vimentin, SNAIL-1, and MMP-9) in HCT-116 and SW-480 cell lines 24 h after SA and CA treatments. Results are presented as the mean ± standard error of three independent experiments. *indicates statistical significance ($p < 0.05$) evaluated by One-way Anova using Tukey post hoc test, treatment *vs*. control

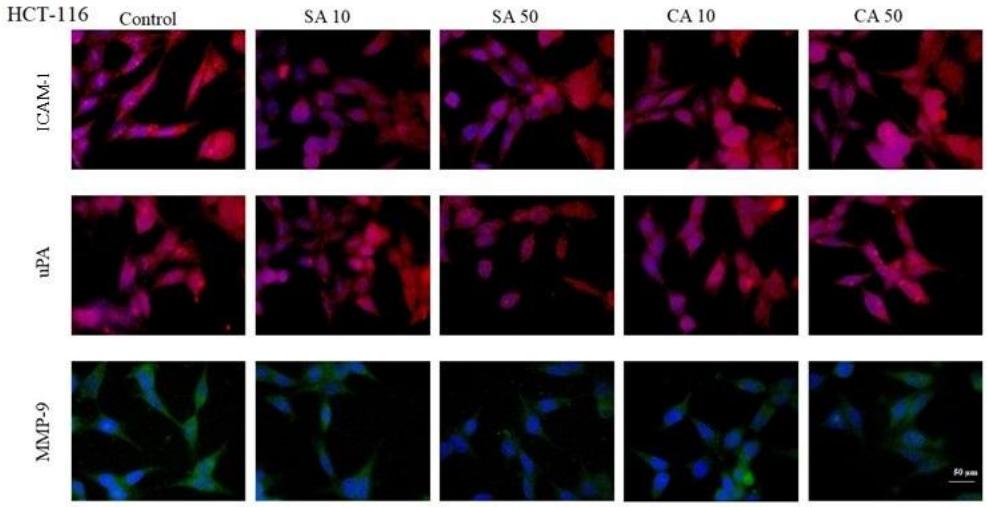

Figure 7.
Representative micrographs of targeted proteins (ICAM-1, uPA, and MMP-9) under the treatments in HCT-116 cells used for relative quantification of expression

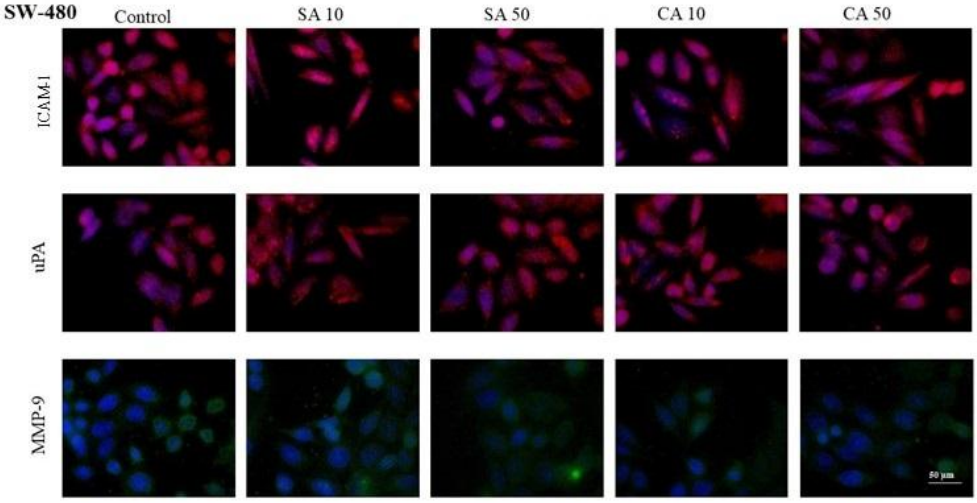

Figure 8.
Representative micrographs of targeted proteins (ICAM-1, uPA, and MMP-9) under the treatments in SW-480 cells used for relative quantification of expression

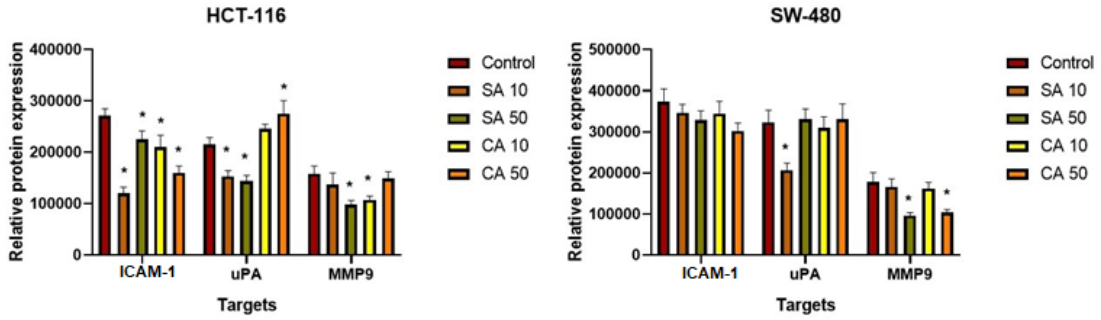

Figure 9.
Relative protein expression of ICAM-1, uPA, and MMP-9 proteins in HCT-116 and SW-480 cell lines 24 h after SA and CA treatments. Results are presented as the mean ± standard error of three independent experiments. *indicates statistical significance ($p < 0.05$) evaluated by One-way Anova using Tukey post hoc test, treatment *vs*. control

Syringic acid modulated the expression of EMT-related markers in a concentration-dependent manner in HCT-116 cells. At lower concentrations, SA induced *SNAIL-1* and *CTNNB-1* expression, suggesting activation of EMT-associated pathways; however, the downregulation of Vimentin and the suppression of MMP-9 indicate that cytoskeletal reprogramming and an invasive phenotype were not achieved (Figure 6). These findings, together with the observed decrease in *NFκB* expression, need to be interpreted in light of NFκB signaling's post-translational regulation. Further functional studies would be required to clarify its role in EMT regulation, while involvement of other signaling pathways, including Wnt/β-catenin (29), PI3K/AKT (30), Hedgehog-GLI, and NOTCH (31), cannot be excluded. These were not directly investigated in this study. In contrast, higher concentrations of SA suppressed *SNAIL-1* and *CTNNB-1* expression while inducing *Vimentin* expression, which may be consistent with stress-associated cytoskeletal remo-deling rather than functional EMT. Importantly, invasion-associated mediators were downregulated at all concentrations, correlating with the observed anti-migratory and anti-invasive effects. Partial EMT involves modulation of EMT-regulating transcription factors (*SNAIL-1, CTNNB-1,* and *NF-κB*), together with partial changes in mesenchymal markers and invasion-related mediators (MMP-9, uPA, and ICAM-1) (32). In contrast, stress-associated cytoskeletal remodeling involves alterations in structural cytoskeletal components typical of mesenchymal cells. Still, it occurs in the absence of activation of EMT transcriptional regulators and invasion-associated functional proteins (33). In SW-480 cells, SA induced upregulation of *SNAIL-1* as a mesenchymal marker at both tested concentrations; however, this was followed by suppression of *CTNNB-1* and invasion-associated proteins ICAM-1, uPA, and MMP-9 (results of protein expression were calculated based on micrographs shown in Figures 7 and 8 and presented in Figure 9). Importantly, inhibition of key proteolytic mediators at the protein level suggests that SA interferes with EMT function, resulting in a less invasive phenotype despite mesenchymal marker induction.

Caffeic acid results showed effects similar to those of SA, with a few differences. Based on the results of the gene expression profile of EMT markers under the influence of CA (Figure 6), the increased *NFκB* gene expression at higher concentrations in the HCT-116 cell line may suggest a higher requirement for NFκB protein. Therefore, further functional assessment of NFκB activity is required to clarify its role in stress responses and pathway activation (34). In addition, in both treatment concentrations on HCT-116 cells, the decrease in CTNNB-1 expression and the increase in pro-invasive VIMENTIN, while simultaneously decreasing SNAIL-1, may indicate partial EMT-like changes but have not yet acquired all the morphological characteristics of mesenchymal cells (32). Secme *et al*. (23) reported significantly decreased MMP-9 gene expression in HCT-116 cells, consistent with our results. On the other hand, SW-480 cells exhibit greater resistance to treatment, and most of the expressed genes suggest a pro-invasive potential. However, regulatory mechanisms in the post-transcriptional or post-translational pathways may contribute to reduced protein expression of key pro-invasive proteins, such as ICAM-1 and MMP-9 (protein expression was quantified from micro-graphs shown in Figures 7 and 8 and presented in Figure 9). Given the limited data on the biological effects of pure CA, comparing it with its more extensively studied derivative, caffeic acid phenethyl ester (CAPE), may provide useful mechanistic insight. Previous studies have demonstrated that CAPE reduces β-catenin expression in SW-480 (35) and HCT-116 cells (36), supporting the notion that CA derivatives may modulate EMT-related signaling pathways. This is the case with our results on the HCT-116 cell line at both concentrations, while

treatment on the SW-480 line showed no significant differences. Also, CAPE reduced MMP-9 expression in hepatocellular carcinoma (37), which is consistent with our results showing lower concentrations of CA treatment reduced MMP-9 expression in both cell lines. CA appears to modulate invasion and EMT markers depending on the cell line tested, potentially inducing partial EMT in HCT-116 cells while suppressing the pro-invasive potential of SW-480 cells at the protein expression level.

Due to uneven expression of EMT markers, we observe partial EMT, characterized by the coexpression of epithelial and mesenchymal markers. Still, cells do not exhibit full invasive capacity, as indicated by functional migration assays (32).

Conclusions

In conclusion, both SA and CA demonstrated weak cytotoxic effects in HCT-116 and SW-480 CRC cells and significantly reduced their invasive and migratory potential. These effects are associated with modulation of key EMT, invasion, and migratory markers, including *SNAIL-1, VIMENTIN, CTNNB-1*, MMP-9, uPA, and ICAM-1. Based on the results for gene and protein expression, it can be concluded that cells undergo partial EMT. These findings indicate activation of some other signaling pathways and time-dependent regulation of transcription factors. Overall, these findings suggest that SA and CA exhibit anti-invasive and anti-migratory properties in the tested CRC lines, providing a basis for additional studies to further confirm their mechanisms of action and therapeutic applicability.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest

References

1. World Health Organization. Global Cancer Observatory [online]. Geneva: WHO; 2023 [cited 2026 Jan 9]. Available from: https://gco.iarc.who.int/media/globocan/factsheets/populations/900-world-fact-sheet.pdf.
2. Zamfir M, Pușcașu A, Hudiță A, Gălățeanu B, Buburuzan A, Irofiei A, et al. Complementing NGS tissue biopsy with plasma derived cfNA analysis as a liquid biopsy for real-time therapy modulation in colorectal cancer patients. Farmacia. 2025;73(4):963-970.
3. Suman S, Kurisetty V, Das TP, Vadodkar A, Ramos G, Lakshmanaswamy R, et al. Activation of AKT signaling promotes epithelial-mesenchymal transition and tumor growth in colorectal cancer cells. Mol Carcinog. 2014;53:e151-160.
4. Piet M, Paduch R. Ursolic and oleanolic acids in combination therapy inhibit migration of colon cancer cells through down-regulation of the uPA/uPAR-dependent MMPs pathway. Chem Biol Interact. 2022;368:110202.
5. Shoari A, Ardalan Ardalan A, Dimesa AM, Coban MA. Targeting invasion: the role of MMP-2 and MMP-9 inhibition in colorectal cancer therapy. Biomolecules. 2025;15:35.
6. Kaufhold S, Bonavida B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res. 2014;33:62.
7. Zhao X, Ma L, Dai L, Zuo D, Li X, Zhu H, et al. TNF-α promotes the malignant transformation of intestinal stem cells through the NF-κB and Wnt/β-catenin signaling pathways. Oncol Rep. 2020;44:577-588.
8. Usman S, Waseem NH, Nguyen TKN, Mohsin S, Jamal A, Teh MT, et al. Vimentin is at the heart of epithelial mesenchymal transition (EMT) mediated metastasis. Cancers. 2021;13(19):4985.
9. Qiu Z, Wang Y, Zhang Z, Qin R, Peng Y, Tang W, et al. Roles of intercellular cell adhesion molecule-1 (ICAM-1) in colorectal cancer: expression, functions, prognosis, tumorigenesis, polymorphisms and therapeutic implications. Front Oncol. 2022;12:1052672.
10. Abotaleb M, Liskova A, Kubatka P, Büsselberg D. Therapeutic potential of plant phenolic acids in the treatment of cancer. Biomolecules. 2020;10(2):221.
11. Pei J, Velu P, Zareian M, Feng Z, Vijayalakshmi A. Effects of syringic acid on apoptosis, inflammation, and AKT/mTOR signaling pathway in gastric cancer cells. Front Nutr. 2021;8:788929.
12. Alam M, Ahmed S, Elasbali AM, Adnan M, Alam S, Hassan MI, et al. Therapeutic implications of caffeic acid in cancer and neurological diseases. Front Oncol. 2022;12:860508.
13. Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Chitta SK. Syringic acid (SA) - a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed Pharmacother. 2018;108:547-557.

14. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.
15. Krzywik J, Mozga W, Aminpour M, Janczak J, Maj E, Wietrzyk J, et al. Synthesis, antiproliferative activity and molecular docking studies of novel doubly modified colchicine amides and sulfonamides as anticancer agents. Molecules. 2020;25(8):1789.
16. Legner J, Jovanović Krivokuća M, Vilotić A, Pirković A, Nacka-Aleksić M, Bojić-Trbojević Ž. Galectin-8 contributes to human trophoblast cell invasion. Int J Mol Sci. 2024;25(18):10096.
17. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. J Vis Exp. 2014;(88):51046.
18. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156-159.
19. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protoc. 2008;3(6):1101-1108.
20. Ćurčić MG, Stanković MS, Cvetković D, Topuzović M, Marković SD. Ligustrum vulgare L.: in vitro free radical scavenging activity and pro-oxidant properties in human colon cancer cell lines. Dig J Nanomater Biostruct. 2014;9(4):1689-1697.
21. Abaza MS, Al-Attiyah R, Bhardwaj R, Abbadi G, Koyippally M, Afzal M. Syringic acid from Tamarix aucheriana possesses antimitogenic and chemo-sensitizing activities in human colorectal cancer cells. Pharm Biol. 2013;51(9):1110-1124.
22. Mihanfar A, Darband SG, Sadighparvar S, Kaviani M, Mirza-Aghazadeh-Attari M, Yousefi B, et al. In vitro and in vivo anticancer effects of syringic acid on colorectal cancer: possible mechanistic view. Chem Biol Interact. 2021;337:109337.
23. Secme M, Mutlu D, Elmas L, Arslan S. Assessing effects of caffeic acid on cytotoxicity, apoptosis, invasion, GST enzyme activity, oxidant, antioxidant status and micro-RNA expressions in HCT116 colorectal cancer cells. S Afr J Bot. 2023;157:19-26.
24. Villota H, Moreno-Ceballos M, Santa-González GA, Uribe D, Castañeda ICH, Preciado LM, et al. Biological impact of phenolic compounds from coffee on colorectal cancer. Pharmaceuticals. 2021;14(8):761.
25. Ghannadi A, Shabani L, Yegdaneh A. Cytotoxic, antioxidant and phytochemical analysis of Gracilaria species from Persian Gulf. Adv Biomed Res. 2016;5:139.
26. Bartmańska A, Tronina T, Popłoński J, Milczarek M, Filip-Psurska B, Wietrzyk J. Highly cancer selective antiproliferative activity of natural prenylated flavonoids. Molecules. 2018;23(11):2922.
27. Park SR, Kim SR, Hong IS, Lee HY. A novel therapeutic approach for colorectal cancer stem cells: blocking the PI3K/Akt signaling axis with caffeic acid. Front Cell Dev Biol. 2020;8:585987.
28. Grada A, Otero-Vinas M, Prieto-Castrillo F, Obagi Z, Falanga V. Research techniques made simple: analysis of collective cell migration using the wound healing assay. J Invest Dermatol. 2017;137(2):e11-e16.
29. Hu C, Ye M, Bai J, Liu P, Lu F, Chen J, et al. Methylmalonic acid promotes colorectal cancer progression via activation of Wnt/β-catenin pathway mediated epithelial-mesenchymal transition. Cancer Cell Int. 2023;23(1):131.
30. Maharati A, Moghbeli M. PI3K/AKT signaling pathway as a critical regulator of epithelial-mesenchymal transition in colorectal tumor cells. Cell Commun Signal. 2023;21:201.
31. Citarella A, Catanzaro G, Besharat ZM, Trocchianesi S, Barbagallo F, Gosti G, et al. Hedgehog-GLI and Notch pathways sustain chemoresistance and invasiveness in colorectal cancer and their inhibition restores chemotherapy efficacy. Cancers. 2023;15(5):1471.
32. Junaid F, Tomic G, Kemp R, Winton DJ. Single-copy SNAIL1 upregulation causes partial epithelial-mesenchymal transition in colon cancer cells. BMC Cancer. 2023;23:153.
33. Leggett SE, Hruska AM, Guo M, Wong IY. The epithelial-mesenchymal transition and the cytoskeleton in bioengineered systems. Cell Commun Signal. 2021;19(1):32.
34. Yang Y, Li Y, Wang K, Wang Y, Yin W, Li L. P38/NF-κB/SNAIL1 pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte. PLoS One. 2013;8(3):e58915.
35. He YJ, Liu BH, Xiang DB, Qiao ZY, Fu T, He YH. Inhibitory effect of caffeic acid phenethyl ester on the growth of SW480 colorectal tumor cells involves β-catenin associated signaling pathway down-regulation. World J Gastroenterol. 2006;12(31):4981-4985.
36. Wang D, Xiang DB, He YJ, Li ZP, Wu XH, Mou JH, et al. Effect of caffeic acid phenethyl ester on proliferation and apoptosis of colorectal cancer cells in vitro. World J Gastroenterol. 2005;11(26):4008-4012.
37. Lee KW, Kang NJ, Kim JH, Lee KM, Lee DE, Hur HJ, et al. Caffeic acid phenethyl ester inhibits invasion and expression of matrix metalloproteinase in SK-Hep1 human hepatocellular carcinoma cells by targeting nuclear factor kappa B. Genes Nutr. 2008;2(4):319-322.