Anticancer effects of the Kiperin Purple Collagen complex: Evidence from HCT116 and MIA PaCa-2 cell models

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Abstract. Purple Collagen is a bioactive complex containing double-hydrolyzed collagens (types I, II, III, V and X) and various bioactive components (inulin, elderberry extract, magnesium citrate and malate, eggshell membrane, bromelain, black cumin extract and liposomal vitamin C). These bioactive compounds have attracted increasing scientific interest due to their ability to modulate key cancer-associated pathways, including the inhibition of cell proliferation and migration, suppression of oxidative stress via free radical scavenging and induction of apoptosis through mitochondrial and caspase-dependent mechanisms. While individual components of the Purple Collagen complex (PCC) have been associated with various health benefits, their combined effects on cancer cell behavior remain largely unexplored. The present study investigated the effects of PCC on cell proliferation, migration, oxidative stress and apoptosis in two cancer cell lines: HCT116 (colorectal carcinoma) and MIA PaCa-2 (pancreas carcinoma). The cell viability, migration, oxidative stress [total oxidant status (TOS)/total antioxidant status (TAS)] and apoptotic and cell cycle regulatory markers (*BAX*, *BCL2*, *TP53* and cyclin-dependent kinase inhibitor 1) were analyzed following treatment with 1 μg/ml PCC. Experiments were performed *in vitro*, and statistical significance was assessed. Cell counts for viability and proliferation analyses were obtained with a Thoma hemocytometer after trypan blue staining. PCC treatment significantly reduced cell proliferation in HCT116 and MIA PaCa-2 cells (P=0.0141 and P=0.0004, respectively). Migration assays demonstrated significant reductions at intermediate time points in both cell lines, HCT116 (P=0.0091) and MIA PaCa-2 cells (P=0.01). TOS and TAS levels revealed a cell-type-specific response, with a marked TAS decrease in HCT116 (P=0.0095). PCC caused an apoptotic and cell cycle regulatory effect, affecting *BCL2* and *p21* expression levels significantly in both cell lines (P<0.05). PCC exhibits notable cell-type-specific effects, inhibiting proliferation and migration in colon and pancreas carcinoma cells while modulating oxidative stress and apoptosis. The findings highlight the potential of bioactive compounds as selective modulators of cancer cell behavior. Further *in vivo* studies are required to evaluate these effects and their clinical relevance.

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Introduction

Cancer is a critical health issue characterized by high rates of morbidity and mortality. Cancer is a major societal, public-health and economic challenge in the 21st century, accounting for ~1 in 6 deaths (16.8%) and nearly 1 in 4 deaths from non-communicable diseases (NCD; 22.8%) worldwide. Cancer contributes to 30.3% of premature NCD deaths among those aged 30-69 years and ranks among the three leading causes of death in this age group in 177 of 183 countries (1). Beyond being a key barrier to increasing life expectancy, cancer incurs substantial societal and macroeconomic costs that vary by cancer type, geography and sex (2). Underscoring the disproportionate burden on women, an estimated one million children became maternal orphans in 2020 due to a maternal cancer death, nearly half attributable to breast or cervical cancer (3). The extracellular matrix (ECM), as a key component of the tumor stroma, notably influences cancer progression by serving as both a physical scaffold and a regulator of cell and tissue functions. Beyond merely transmitting signals, the ECM also generates biochemical and biophysical cues that activate cellular responses (4). The interaction between tumor cells and the ECM is dynamic and reciprocal, leading to continuous reshaping of the surrounding malignant tissue. Research indicates that tumors can exploit ECM remodeling to establish a microenvironment conducive to tumorigenesis and metastasis (5). In turn, cellular behaviors such as adhesion, migration, angiogenesis and malignant transformation are influenced by changes in the ECM (6,7). Within this intricate relationship between the ECM and tumor cells, collagens serve a pivotal role. As the primary component of the ECM, collagens constitute ~30% of its structure, with 28 distinct collagen types identified. These various collagens

contribute to the formation of the basal membrane and interstitial matrix, creating tissue-specific ECM compositions (8). Alterations in collagen within the tumor microenvironment generate biomechanical signals that are detected by both tumor and stromal cells, initiating a series of biological processes. A previous study has highlighted the abnormal behaviors of collagens in cancer progression, including degradation, remodeling, fragmentation, linearization and fasciculation (9), demonstrating the role of collagens in precancerous lesions and cancer development. Understanding the mechanisms by which collagens influence different stages of cancer progression has enhanced their diagnostic and prognostic value while opening new avenues for therapeutic target identification (10).

Several antioxidants, anti-inflammatory and antimicrobial compounds targeting the synthesis and function of reactive oxygen species (ROS) and inflammatory processes are already available as anticancer agents. These compounds offer notable advantages over traditional chemotherapeutic drugs by precisely targeting cancerous processes while minimizing harm to healthy cells (11). The Purple Collagen complex (PCC) incorporates several bioactive compounds known for their diverse health benefits, including inulin which is a prebiotic fiber that promotes gut health by stimulating the growth of beneficial intestinal bacteria (12). Another compound is the elderberry, which is derived from *Sambucus nigra*, rich in flavonoids, particularly anthocyanins, which exhibit antioxidant properties. These compounds support immune function and have been traditionally used to alleviate cold and flu symptoms (13). Another plant-based compound is the black cumin extract sourced from *Nigella sativa*, which is known for its antioxidant, anti-inflammatory and antimicrobial effects, attributed to its active component, thymoquinone (14). Magnesium citrate and malate found in PCC are highly bioavailable. Magnesium citrate is commonly used to treat constipation and improve bone health, while magnesium malate has been suggested to benefit individuals with fibromyalgia (15). The eggshell membrane contains naturally occurring glycosaminoglycans and proteins that support joint health by reducing pain and stiffness associated with joint disorders (16). Another compound found in the complex is the bromelain, an enzyme extracted from pineapples, bromelain possesses anti-inflammatory and proteolytic properties, aiding in digestion and potentially reducing inflammation (17). The final ingredient of PCC is the liposomal vitamin C which is a potent antioxidant that supports immune function and skin health. Encapsulating vitamin C in liposomes enhances its bioavailability, ensuring efficient delivery and absorption (18). Collectively, these components contribute to the multifaceted health-promoting properties of PCC.

Due to the established links between the bioactive compounds and cancer dynamics, the present study aimed to examine *in vitro* effects of Purple Collagen, a complex containing bioactive double hydrolyzed collagens type I, II, III, V and X collagen peptides and several antioxidant and anti-inflammatory agents (inulin, elderberry extract, magnesium citrate and malate, eggshell membrane, bromelain, black cumin extract and liposomal vitamin C), on cell viability, migration, oxidative stress, antioxidant status and apoptotic markers of two cancer types HCT116 colon carcinoma and MIA PaCa-2 pancreas carcinoma cells. By evaluating the mechanisms through which PCC influences these processes, the present study aimed to uncover its potential as a therapeutic agent in these cancer types.

Materials and methods

Cell culture. The study protocol was approved by Scientific Research Ethical Committee of Biruni University in May 2024 with an approval number of 2024-BIAEK/01-41. HCT116 colon carcinoma cells were obtained from ATCC (cat. no. CCL-247) and MIA PaCa-2cells obtained from ATCC (cat. no. CRL-1420). The present study includes the MIA PaCa-2 pancreatic carcinoma cell line and the HCT116 cell line, both of which are of human origin.

All cell lines were cultured in DMEM (Nutriculture; EcoTech Biotechnology Inc.) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics (Diagnovum) at 37˚C in a humidified incubator containing 5% CO_2 (Kabin Incubator; Esco Lifesciences Group). The cells were maintained in these conditions throughout the experiments.

Purple Collagen, the commercial test compound used in the present study, was supplied by the company Kiperin Pharmaceutical Food Industry and Trade Limited Company. Kiperin® PCC contains 10 g collagen (bioactive double hydrolyzed collagens containing type I, II, III, V and X collagen peptides derived from grass-fed, pasture-raised calves), 3 g of inulin, 500 mg of elderberry (*Sambucus*) extract, 180 mg magnesium citrate (providing 29 mg magnesium), 180 mg magnesium malate (providing 27 mg magnesium), 100 mg eggshell membrane, 90 mg bromelain (2,400 gelatin-digesting units), 90 mg black cumin extract, 90 mg liposomal vitamin C and 40 mg 100% natural blueberry flavor. The dosage was determined in accordance with the Turkish Ministry of Agriculture and Forestry's regulatory standards, which specify that dietary supplements containing hydrolyzed collagen should not provide >10 g per day (19). A concentration was used similar to a previous double hydrolyzed collagen study, which positively affected healthy cells and suppressed cancer cells (20), and the dosage was determined accordingly. The selected doses, 0.5, 1 and 1.5 μg/ml, were normalized to the *in vitro* cell number ($6x10^4$ cells/well) to approximate physiologically attainable levels while minimizing off-target cytotoxic responses. Due to the structural and functional similarities between hydrolyzed collagen and purple collogen, the same dosage range in the experiments was applied. Cells were detached with 0.25% trypsin-EDTA, neutralized with medium containing 10% FBS, pelleted at 300 x g for 5 min at 37˚C, and resuspended in PBS. The suspension was mixed 1:1 with 0.4% trypan blue, incubated for 2-3 min at room temperature, and then 10 μl was loaded onto a Thoma hemocytometer. Cells were counted under a light microscope (10X objective) in the four large corner squares, including cells touching the top and left borders. Counts from both chambers were averaged. Viable (unstained) and non-viable (blue) cells were recorded separately; cell density was calculated as cells/ml=(mean cells per square) x dilution factor (2) x 10^4, and % viability=viable/(viable + non-viable) x100. The resulting density was used to adjust seeding for subsequent assays.

The effective dose of Purple Collagen was determined as 1 μg/ml concentration among three doses tested (0.5, 1 and

1.5 μg/ml) by cell counts measured for three times using the Mindray BC-6800 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.) cell counter. Each cell line was divided into two experimental groups: Control cells (untreated) and collagen-treated cancer cells (1 μg/ml concentration of Purple Collagen). Cells were incubated with the respective treatments for 48 h at 37˚C.

Wound healing assay: To evaluate cell migration, a wound healing assay was performed in DMEM supplemented with 10% FBS, consistent with previous studies utilizing HCT116 and MIA PaCa-2 cell lines (21-23). At least 90% confluent monolayers of cells were scratched with a sterile pipette tip, creating a uniform gap. Images of wound closure of HCT116 and MIA PaCa-2 cells were taken at 0, 6, 15, 30 and 43 h using an inverted light microscope (Inverted ICX41 SOPTOP; China) at the same marked position. Wound healing was quantified by measuring the percentage of wound closure over time (24). Wound closure area was measured using ImageJ (1.54g; National Institutes of Health) software.

Measurement of oxidative stress levels and antioxidant capacity. Cell lysates were prepared from HCT116 and MIA PaCa-2 cell lines following standard protocols (25,26) and stored at -80˚C until analysis. All reagents and samples were equilibrated to room temperature before use.

The total oxidant status (TOS) and total antioxidant status (TAS) levels in two cell lines were assessed using commercial assay kits provided by Rel Assay Diagnostics. Both assays were performed according to the manufacturer's protocol, utilizing a spectrophotometer (Epoch Microplate Spectrophotometer; BioTek; Agilent Technologies, Inc.) to quantify the absorbance changes associated with the oxidative and antioxidative properties of the samples.

To measure TOS, the assay relied on the oxidation of ferrous ions to ferric ions by oxidant molecules in the sample. The ferric ions formed a colored complex with a chromogen in an acidic medium, and the color intensity was proportional to the total oxidant molecules present. For the assay, 45 μl cell lysate, standard solution or distilled water (used as a blank) was mixed with 300 μl buffer solution (pH 1.75). The absorbance of the reaction mixture was first read at 530 nm after 30 sec. Subsequently, 15 μl ferrous ion solution was added to the mixture, and after incubation for 5 min at 37˚C or 10 min at room temperature, the absorbance was read again. The TOS was calculated based on the change in absorbance and expressed as μmol H_2O_2 equivalents (Eq)/l.

For TAS measurements, antioxidants in the sample reduced dark blue-green ABTS radical cations to their colorless form. The assay used 18 μl cell lysate, standard solution or distilled water (blank), which was mixed with 300 μl acetate buffer (pH 5.8). The initial absorbance of the reaction mixture was measured at 660 nm after 30 sec. Subsequently, 45 μl ABTS prochromogen solution was added, and after incubation for 5 min at 37˚C or 10 min at room temperature, the final absorbance was recorded. The antioxidant capacity was quantified based on the change in absorbance and expressed as mmol Trolox Eq/l.

The TOS and TAS levels were calculated and analyzed statistically, with results expressed as mean ± standard deviation (SD).

Reverse transcription (RT)-quantitative (q)PCR for apoptotic and cell cycle regulatory markers expression. Total RNA was extracted from cells, and the concentration was assessed using a Qubit fluorometer. Subsequently, 500 ng RNA was reverse transcribed into complementary DNA (cDNA) using the OneScript® Plus cDNA Synthesis Kit (Applied Biological Materials Inc.) according to the manufacturer's instructions. The RT reaction was carried out with Moloney-Murine Leukemia Virus reverse transcriptase. The commercial kit used in the present study was applied according to the manufacturer's instructions. Internal quality control steps, including positive and negative control samples, were performed to validate the reliability and specificity of the assay. These controls were included in every experimental run. For experimental validation, PCR amplification was performed, followed by agarose gel electrophoresis. The observation of a single band of the expected size confirmed that each primer pair was specific and effective.

RT-qPCR was performed using the BlasTaq™ 2X qPCR MasterMix (Applied Biological Materials Inc.) following the supplier's protocol. Each reaction contained 2 μg of cDNA template, 1 μM each primer, and the appropriate volume of MasterMix, adjusted to a final volume of 25 μl with nuclease-free water. The primers used were as follows: *BAX*, forward 5'-GCCCTTTTGCTTCAGGGTTTCA-3' and reverse 5'-CTGTCCAGTTCGTCCCCGAT-3'; *BCL2* forward 5'-GTG GATGACTGAGTACCT-3' and reverse 5'-CCAGGAGAA ATCAAACAGAG-3'; *TP53* forward 5'-AATCTCCGCAAG AAAGGGGAG-3' and reverse 5'-TTGGGCAGTGCTCGC TTAG-3'; cyclin-dependent kinase inhibitor 1 (p21) forward 5'-GACTGTGATGCGCTAATGGC-3' and reverse 5'-CCG TGGGAAGGTAGAGCTTG-3'; *GAPDH* (housekeeping gene) forward 5'-CCACCCATGGCAAATTCC-3' and reverse 5'-TGGGATTTCCATTGATGACAAG-3'.

Amplification and detection were performed on a Bio-Rad CFX96™ Real-Time PCR System (Bio-Rad Laboratories, Inc.) under the following cycling conditions: Initial denaturation at 95˚C for 3 min, followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 60˚C for 15 sec and extension at 72˚C for 15 sec. Fluorescence data were collected at the end of each extension step. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method, normalizing the gene expressions to *GAPDH* as the internal control (27).

Statistical analysis. All statistical analyses were performed using GraphPad Instat Software 8.0.1 (Dotmatics). The distribution of the data was assessed for normality using the Kolmogorov-Smirnov test. For data that followed a normal distribution, comparisons between groups were performed using an unpaired t-test. For non-normally distributed data, a Mann-Whitney U test was applied. $P<0.05$ was considered to indicate a statistically significant difference. To ensure the objectivity and integrity of the findings, all statistical analyses were performed by an independent researcher not affiliated with Kiperin Pharmaceutical.

Results

Cell viability and proliferation. The cell numbers of HCT116 and MIA PaCa-2 cell lines were assessed in the control

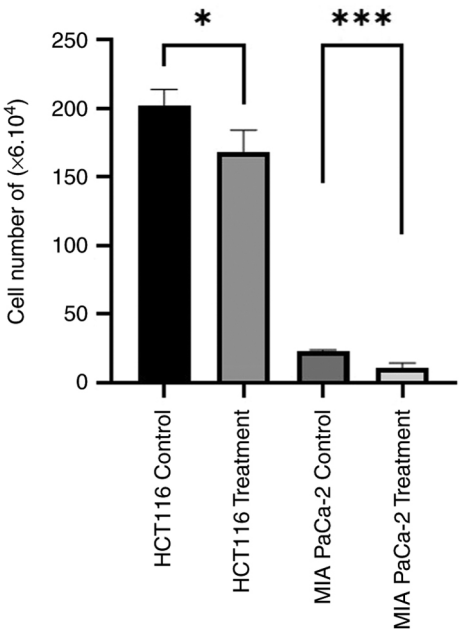

Figure 1. Effect of 1 μg/ml Kiperin Purple Collagen Complex treatment on cell proliferation in HCT116 and MIA PaCa-2 cell lines. Bar graphs represent mean ± standard deviation of three independent experiments. *P<0.05, ***P<0.01 vs. control group. Treatment, 1 μg/ml Kiperin Purple Collagen complex; Control, untreated.

conditions and after treatment with 1 μg/ml dose of PCC (Fig. 1). The results indicate a significant decrease in the number of HCT116 cells treated with the PCC compared with the control group (P=0.0141). Similarly, a significant reduction in the number of MIA PaCa-2 cells was observed following treatment with the PCC compared with the control group (P=0.0004). These findings suggest that treatment with PCC has inhibitory effects on the proliferation of colon cancer and pancreas carcinoma cells.

Cell migration. The migration rates of HCT116 and MIA PaCa-2 cells over different incubation periods are presented in Table I, and representative microscopic images are shown in Figs. 2 and 3. In both cell lines, treatment with 1 μg/ml PCC reduced migration compared with the controls, although the extent and timing of inhibition varied between the two cell lines.

For MIA PaCa-2 cells, migration was significantly reduced in the PCC group at 6 h (5.0 vs. 7.8% P=0.006) and at 43 h (72.1 vs. 91.7%; P=0.002). However, at 15 and 30 h, the reductions (43.8 vs. 46.1% and 42.3 vs. 74.0%, respectively) did not reach statistical significance (P=0.549 and P=0.226, respectively).

For HCT116 cells, PCC treatment resulted in consistently significant reductions in migration at early and intermediate time points. At 6 h, migration was markedly higher in treated cells (12.8 vs. 1.6%; P=0.00014), compared with the control cells. At 15 and 30 h, PCC-treated cells showed significantly lower migration compared with the controls (30.4 vs. 43.3%; P=0.0003 ; 61.2 vs. 63.8%; P=0.003). At 43 h, the control cells had achieved complete closure (100%), while treated cells reached 85.9%; however, this difference was not statistically significant (P>0.999).

Table I. Migration rate of the wound healing assay in control or PCC-treated cells.

Incubation	Migration rate of MIA PaCa-2 cells (%)	Migration rate of HCT116 cells (%)
6 h		
Control	7.8	1.6
1 μg/ml PCC	5.0	12.8
P-value	0.006	<0.00014
15 h		
Control	46.1	43.3
1 μg/ml PCC	43.8	30.4
P-value	0.549	<0.0003
30 h		
Control	74.0	63.8
1 μg/ml PCC	42.3	61.2
P-value	0.226	0.003
43 h		
Control	91.7	100
1 μg/ml PCC	72.1	85.9
P-value	0.002	>0.999

PCC, Purple Collagen complex.

Oxidative and antioxidant status. The TOS and TAS levels in HCT116 and MIA PaCa-2 cells under control conditions and after treatment with 1 μg/ml PCC are presented in Table II. For HCT116 cells, TOS levels did not differ significantly between the control (0.302±0.181 μmol H_2O_2 Eq/l) and PCC-treated groups (1.253±2.401 μmol H_2O_2 Eq/l; P=0.7619). However, TAS levels demonstrated a significant reduction in the PCC-treated group (0.122±0.102 mmol Trolox Eq/l) compared with the control (2.414±2.39 mmol Trolox Eq/l; P=0.0095).

For MIA PaCa-2 cells, no significant differences were observed in either TOS levels (control, 5.09±0.56 μmol H_2O_2 Eq/l; PCC-treated, 8.72±2.45 μmol H_2O_2 Eq/l; P=0.292) or TAS levels (control, 2.300±0.004 mmol Trolox Eq/l; PCC-treated, 0.007±0.000 mmol Trolox Eq/l; P=0.686). These results indicate that 1 μg/ml PCC has a cell-specific effect on oxidative and antioxidant balance; however, there was a significant decrease in TAS levels increase in TOS levels in HCT116 and MIA PaCa-2 cell lines.

Expression levels of apoptotic and cell cycle regulatory markers. The expression levels of *BAX*, *BCL2*, *TP53* and *p21* in HCT116 and MIA PaCa-2 cells under control conditions and after treatment with 1 μg/ml PCC are presented in Fig. 4. In both cell lines, *BAX* expression was not significantly altered with PCC treatment compared with the control. *BCL2* expression was significantly decreased in MIA PaCa-2 cells (P=0.00029), but not in HCT116 cells. The tumor suppressor marker *p21* showed significant upregulation in HCT116 cells (0.00221) after PCC treatment, but downregulation in MIA PaCa-2 cells (P=0.00025). *TP53* expression did not differ with PCC treatment in both cell lines compared

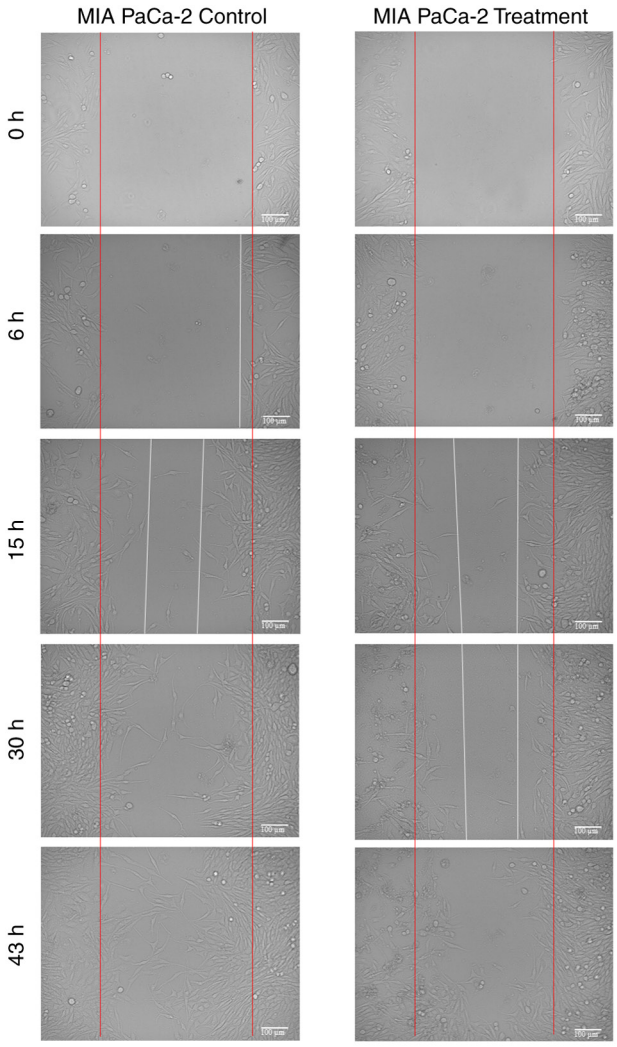

Figure 2. Representative images of the wound healing assay of untreated or Kiperin Purple Collagen complex-treated MIA PaCa-2 cells.

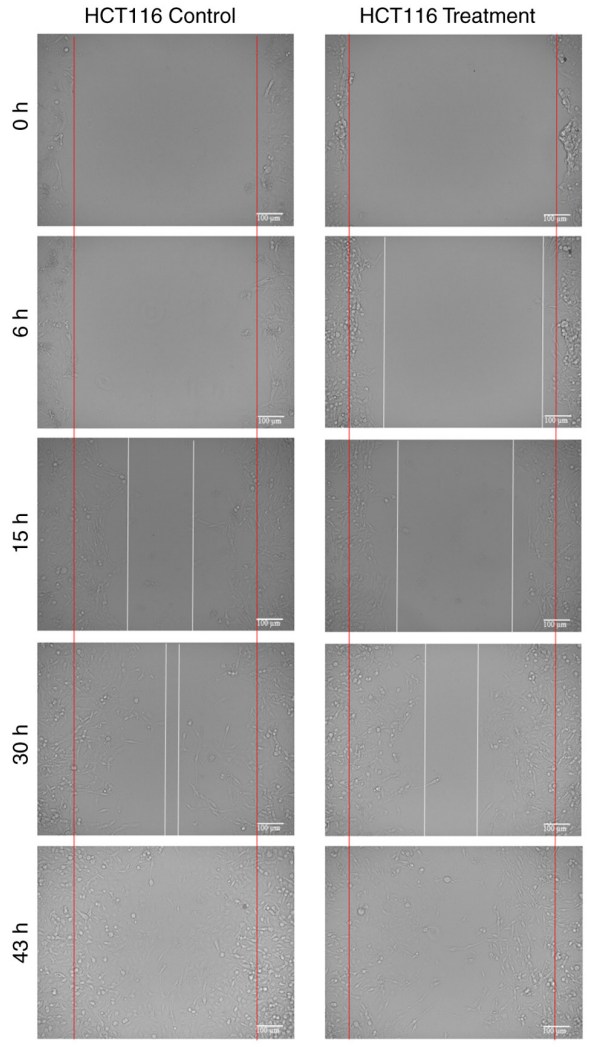

Figure 3. Representative images of the wound assay of untreated or Kiperin Purple Collagen complex-treated HCT116 cells.

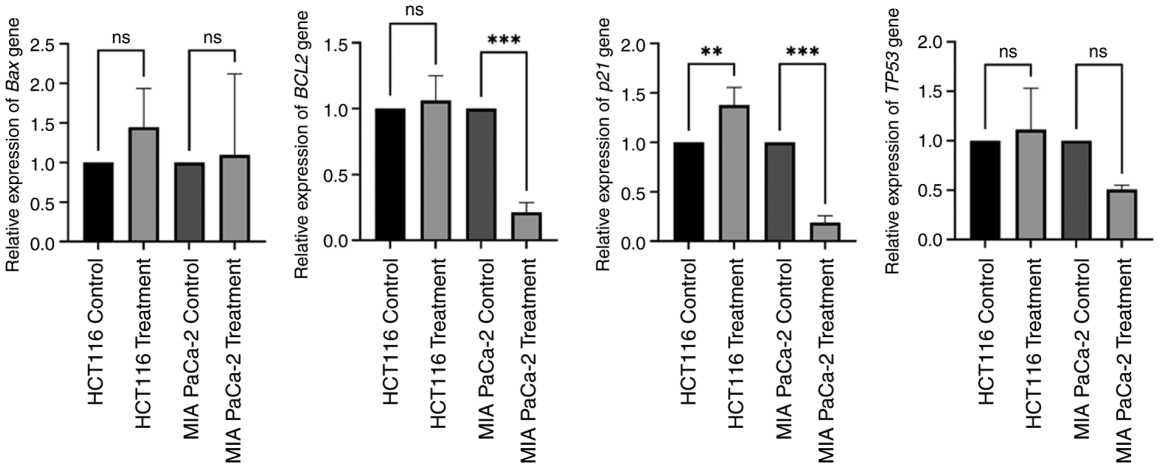

Figure 4. Expression levels of *BAX*, *BCL2*, *TP53* and *p21* in HCT116 and MIA PaCa-2 cell groups treated with 1 μg/ml Purple Collagen Complex from three independent experiments. Data presented as mean ± standard deviation of three independent experiments. **P<0.01, ***P<0.001 vs. control group. ns, non-significant; Treatment, 1 μg/ml Kiperin Purple Collagen complex; Control, untreated.

with the control. These results suggest that 1 μg/ml PCC caused an apoptotic and cell cycle regulatory effect, affecting *BCL2* and *p21* expression levels in different cell lines. It was observed that pro-apoptotic pathways were activated in both the HCT116 and MIA PaCa-2 cell lines. This highlights the potential role of the PCC in modulating apoptosis and cell cycle regulation.

Table II. TOS and TAS levels in HCT116 and MIA PaCa-2 cells treated with 1 μg/ml PCC.

A, HCT116 cells

Group	TOS (μmol H_2O_2 Eq/l)	TAS (mmol Trolox Eq/l)
Control	0.302±0.181	2.414±2.390
1 μg/ml PCC	1.253±2.401	0.122±0.102
P-value	0.762	0.01

B, MIA PaCa-2 cells

Group	TOS (μmol H_2O_2 Eq/l)	TAS (mmol Trolox Eq/l)
Control	5.090±0.560	2.300±0.004
1 μg/ml PCC	8.720±2.450	0.007±0.00014
P-value	0.292	0.600

TOS, total oxidant status; TAS, total antioxidant status; Eq, equivalents; PCC, Purple Collagen complex.

Discussion

The findings of the present experimental study demonstrate that the PCC exhibits significant cell-type-specific effects on cancer cell proliferation, migration, oxidative stress and the expression of apoptotic and cell cycle regulatory markers. The observed reduction in cell viability and proliferation in HCT116 and MIA PaCa-2 cells suggests that PCC treatment selectively inhibits the growth of colon cancer and pancreas carcinoma cells. Furthermore, the differential modulation of oxidative and antioxidant status, with a notable reduction in TAS levels in HCT116 cells, highlights the potential of PCC to influence cellular redox balance. The significant reduction in migration observed in both cell lines, particularly at intermediate time points, further underscores the potential role of PCC in inhibiting cancer cell motility and metastatic capacity.

Colorectal cancer is the second leading cause of cancer-associated mortalities globally (28). While approximately half of patients with colorectal cancer initially respond positively to conventional therapies (29), long-term survival remains poor due to the high likelihood of metastasis following multimodal treatment (30). Therefore, alternative therapies are needed. The treatment of HCT116 colorectal cancer cells with PCC, which includes bioactive double-hydrolyzed collagens (types I, II, III, V and X) and various bioactive components (inulin, elderberry extract, magnesium citrate and malate, eggshell membrane, bromelain, black cumin extract, liposomal vitamin C), resulted in a significant reduction in cell proliferation and migration. This inhibitory effect may be associated with downregulation of anti-apoptotic markers *BCL2* and *p21*. These findings align with existing literature indicating that certain bioactive compounds can modulate apoptotic pathways in colorectal cancer cells (31). For instance, a previous study has demonstrated that targeting specific molecular pathways can enhance the expression of pro-apoptotic signals, thereby promoting cancer cell death (32). Additionally, the observed modulation of oxidative and antioxidant status, evidenced by changes in TOS and TAS levels, suggests that components of the PCC may influence the redox balance within HCT116 cells. This is consistent with research indicating that alterations in oxidative stress can impact cancer cell behavior (33,34). Collectively, these results suggest that PCC exerts its anticancer effects through the modulation of apoptosis, oxidative stress and cell migration pathways in HCT116 colorectal cancer cells.

Sato and Seiki (35) also suggest that hydrolyzed collagen fragments may trigger intracellular stress responses, potentially explaining the upregulation of tumor suppressor proteins such as *TP53* and *p21* in the present study. According to Madri and Furthmayr (36), the interaction of collagen with cellular signaling pathways may affect apoptosis and cell cycle regulation. Bioactive peptides in the PCC may modulate these pathways, leading to the observed changes in apoptotic and cell cycle markers. Blueberry extract, a dietary phytochemical, has been reported to have a chemo-preventive activity in triple negative breast cancer cell lines *in vitro* and *in vivo* (37,38). Blueberry reduced cell proliferation in HCC38, HCC1937 and MDA-MB-231 cells and reduced the metastatic potential of MDA-MB-231 cells through modulation of the PI3K/AKT/NFkB pathway. Bilberry treatment decreased *MMP-9* activity and urokinase-type plasminogen activator secretion, and increased tissue inhibitor of *MMP-1* and plasminogen activator inhibitor-1 secretion in MDA-MB-231 cells (38,39). Minker *et al* (40) reported that the proanthocyanidins extracted from 11 berry species including blueberry may be preventive against human colorectal cancer cell lines by inducing apoptosis. In line with the literature, the PCC, which also contains blueberry extract, may modulate the metastatic features of the cell lines HCT116 and MIA PaCa-2 with different incubation durations.

Notably, the inhibitory effect on migration, observed in both HCT116 and MIA PaCa-2 cells at intermediate time points, aligns with prior studies suggesting that hydrolyzed collagen fragments can interfere with ECM integrity, integrin signaling, and MMP activity, and the phytochemicals with anticancer features. These findings emphasize the complexity of PCC which has potential role against the cancer progression and highlight the potential therapeutic value of bioactive compounds in complex, in selectively modulating cancer cell behavior. Future studies should explore the mechanistic underpinnings of these effects, with a focus on optimizing collagen formulations and dosages to enhance their anticancer efficacy across diverse tumor types.

The findings showed a significant decrease in *BCL2* gene expression in MIA PaCa-2 cells as well as modulation of *p21* expression in both cell types treated with PCC. These changes indicate that although the collagen complex regulates *p21* gene expression, which serves an important role in the repair mechanism and the process leading to apoptosis, it indicates its pro-apoptotic and cell cycle regulatory effect by decreasing *BCL2* gene expression. Mammoto *et al* (41) and Payne and Huang (42) describe how specific collagen interactions can modulate intracellular signaling pathways, including those regulating apoptosis. Hydrolyzed collagen fragments in the PCC can disrupt essential survival pathways by interfering

with ECM-integrin signaling, resulting in increased pro-apoptotic markers. Furthermore, Yin *et al* (43) identified collagen genes associated with epithelial-mesenchymal transition and immune infiltration, highlighting their role in pancreatic carcinoma progression. The observed decrease in *BCL2* expression may indicate that the PCC modulates stress response pathways associated with these processes, pushing pancreatic carcinoma cells towards apoptosis rather than proliferation.

The present study has several limitations that should be considered. The *in vitro* design does not fully replicate the complexity of the tumor microenvironment, including interactions with immune cells and vascular structures. Furthermore, only a single dose of PCC was tested, and a broader dose-response analysis was not performed. The short-term nature of the experiments does not account for potential long-term effects or resistance mechanisms. While significant changes in apoptotic and cell cycle markers were observed, the specific molecular mechanisms underlying these effects were not explored in detail. Future studies are warranted to confirm the observed gene expression changes at the protein and functional levels. Additionally, *in vivo* experiments were not performed, which limits the translational relevance of the results. The study also did not isolate the contributions of individual bioactive components within the collagen complex or assess detailed oxidative stress markers beyond TOS and TAS levels. As the formulation contains multiple bioactive ingredients, it remains unclear whether the observed biological effects are due to individual components or synergistic interactions. Future studies are warranted to investigate the specific contributions and potential synergistic mechanisms of each component A more comprehensive migration analysis and consideration of cell line variability would further strengthen the findings. Addressing these limitations in future research will provide more robust and clinically relevant insights.

In conclusion, the findings of the present study underscore the cell-type-specific effects of PCC on cancer cell proliferation, migration, oxidative stress and apoptosis. While the complex demonstrated significant inhibitory effects on the viability and migration of HCT116 colorectal cancer cells, its effects on MIA PaCa-2 cells varied, reflecting the distinct biological characteristics of these cancer types. The pro-apoptotic and cell cycle-regulatory effects, marked by upregulation of *BCL2* and *p21*, suggest that PCC may modulate key molecular pathways to promote apoptosis in certain cancers. Oxidative and antioxidant modulation, particularly TOS decrease and TAS increase in cancer cells, highlights the role of collagen in influencing redox balance.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LKB served as the project manager, supervised the study, contributed to the data analysis and interpretation, and participated in the manuscript writing and editing. CY performed the statistical analysis, optimized the methodology and provided critical revisions to the manuscript. NH contributed to data interpretation, literature review and manuscript editing. LKB and CY confirm the authenticity of all the raw data. All authors participated in an independent and impartial evaluation of the study. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Scientific Researches Ethical Committee of Biruni University in May 2024 with an approval number of 2024-BIAEK/01-41. All procedures were performed in accordance with ethical research guidelines.

Patient consent for publication

Not applicable.

Competing interests

LKB serves as a scientific consultant for Kiperin Pharmaceutical Food Industry and Trade Limited Company (Istanbul, Turkey). The authors used Kiperin collagen in the study from Kiperin Pharmaceutical Food Industry and Trade Limited Company, although the study was conducted independently, and the company had no role in the study design, data collection, data analysis, interpretation of results or manuscript preparation. The other authors declare that they have no competing interests.

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