RESEARCH ARTICLE

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Anticancer Properties of Garlic and Ginger Extract in Colon Cancer Cell Line

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Abstract

Colon cancer typically affects older adults, though it can happen at any age. Colon cancer, also known as Caco-2, is caused by multiple epigenetic alterations and involves unregulated proliferation, differentiation, and invasion of neighboring tissues. Colon cancer patients have had surgery, radiation, hormone therapy, and chemotherapy. This study investigates a new experimental method using inexpensive and environmentally friendly Egyptian plant extracts. DMSO-dissolved ginger, garlic, cinnamon, and chamomile were employed in this investigation. HPLC and GC-MS were used to analyze plant extracts. These extracts were tested for colon cancer efficacy using various methods. These methods included Caco-2 cells, MTT test, Annexin V-FITC flow cytometry, qRT-PCR, and ELISA. Garlic and ginger were found to be cytotoxic to Caco-2 cells. Compared to cinnamon and chamomile extracts, garlic and ginger have boosted LDH synthesis significantly. Garlic and ginger also altered autophagy genes (Bectin1, Atg5, PTEN) and Caspase-3 expression pathways on proapoptotic signaling. Garlic and ginger increased cleaved PTEN and caspase-3 and decreased Atg5 and Bectin1. Ginger and garlic caused extrinsic apoptosis and prevented Atg5 and Bectin1 phosphorylation. The average IL-8 and IL-6 levels increased significantly after 24 hours, according to ELISA. In conclusion, garlic and ginger extracts modify pro-inflammatory cytokines. Alternative herbal remedies like garlic and ginger may be effective and safe colon cancer treatments.

Keywords: Garlic- Ginger- chamomile- cinnamon- Bectin1- Atg5- PTEN- IL-6- IL-8

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Introduction

The global 2020 colorectal cancer death toll was 940,000, with 1.93 million new cases. 3.2 million additional colorectal cancer cases are expected by 2040 [1]. The five-year survival rate for this cancer is 64.4% [2]. The WHO reported 10 million cancer deaths in 2020, making it the top cause of death [3]. Colorectal cancer is the sixth most frequent disease in Egypt, affecting 3.47% of men and 3% of women [4]. Lack of screening in young people and lifestyle factors, including obesity and nutrition, are the main causes of colorectal cancer [5]. Colorectal cancer molecular alterations fall into three categories: CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and chromosomal instability [6].

Cancer therapies include targeted hormones, radiation, chemotherapy, immunotherapy, and surgery [7]. Peeling, itching, redness, and pain after radiation therapy can make skin wet and infected [8]. Researchers have used in vitro and in vivo cancer models from many sources to test the chemo-therapeutic and chemo-preventive effects of dietary phytochemicals [9-12]. Many nutrients can stop angiogenesis, metastasis, cancer cell invasion, and tumor formation [13].

Natural compounds could replace less hazardous and cheaper medicines [14]. Phytochemicals are different bioactive substances found in plants. They include phytoestrogens, stanols, phenolic acids, terpenes, flavonoids, carotenoids, and phytoestrogens. Flavonoids,

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present in fruits, vegetables, mushrooms, chocolates, teas, and wines, may inhibit cancer cell proliferation. [15, 16]. Garlic, soybeans, tofu, grapes, plums, and berries contain phytoestrogens, which may reduce the risk of colon cancer, menopausal symptoms, heart disease, and osteoporosis [17]. They may prevent cellular damage from free radical oxidation by stimulating anti-inflammatory pathways [18]. Garlic and ginger have been used in culinary and therapeutic ways since ancient times. Ginger extract inhibits colon cancer cell proliferation and causes G2/M phase and death [19]. Research shows that garlic slows cellular growth, promotes apoptosis, and arrests the cell cycle in stomach cancer at G0/G1 [20].

We know that Autophagy has two functions and that inhibiting or inducing autophagic pathways can help prevent and treat cancer [21]. ATG proteins are necessary for autophagosome formation and represent the basic molecular mechanism of Autophagy [22]. The tagged protein assembles during autophagosome expansion or completion to produce two ubiquitin-like conjugation systems. Atg8/LC3-PE and Atg12-Atg5-Atg16L [23]. In yeast, Atg5 and Atg12 form a complex that controls LC3 processing and autophagosome formation [24]. PTEN, a tensin and phosphatase homolog, blocks the PI3K pathway by dephosphorylating phosphatidylinositol (P.I.) 3,4,5-triphosphate into PI-4,5-bisphosphate in 10q23.31. Mutated PTEN is found in 3.86% of CRC patients [6, 25].

Multiple cancer forms show IL-6 and IL-8's pro-oncogenic capabilities. IL-6 promotes tumorigenicity by blocking apoptosis and increasing the proliferation of STAT proteins. Many experimental and clinical studies have linked spontaneous and inflammation-related colorectal cancer (CRC) to the pleiotropic cytokine interleukin-6 (IL-6), which is expressed more in colon cancer and regulates proliferative responses and cell death [26]. The tumor region had increased IL-8, a key marker in CRC. The Cysteine-X-Cysteine Receptor (CXCR) binds and causes cancer in many ways [27]. Intercellular inducers can create a milieu that amplifies the tumor activity of IL-8 through proliferation, angiogenesis, and migration [28, 29], all of which are regulated by both typical and atypical cells, including tumor-associated macrophages (TAMs) [31] and cancer cells [30-32]. A scientific foundation supports chemical and pharmacological anti-colorectal cancer research. And thorough data are needed. This study investigates how natural products, and their bioactive elements can manage and prevent colorectal cancer (CRC) cell lines through diet. Ginger, garlic, chamomile, and cinnamon extracts were compared in our study. We measured the expression of autophagy genes Atg5, PTEN, Beclin-1, and casp3 in Caco-2 cells.

Materials and Methods

Plant extraction

For the plant extraction, 10 mg of garlic, cinnamon, chamomile, and ginger smooth powder were thoroughly mashed, sterilized with 75% ethanol, and air-dried at room temperature. Each sterilized extract was diluted in 1 ml dimethylsulfoxide to 100 g/l. The final extract was incubated until usage at 4°C. GCMS and HPLC tests used plant extracts from UGC Pharma Co. in Badr City, Cairo Governorate, Egypt.

Tests and instruments

The fine chemical content of ginger, garlic, chamomile, and cinnamon ethanol extracts was studied.

GC-MS analysis

A Trace GC-TSQ Mass Spectrometer (Thermo Scientific, Austin, Tx, USA) with TG-5MS direct capillary column analysed ginger, garlic, chamomile, and cinnamon liquids. Comparing mass spectra using WILEY 09 and NIST 14 databases identified components.

HPLC analysis

HPLC measured ginger, garlic, chamomile, and cinnamon solutions [33]. Agilent 1100 HPLC has a UV/V detector and quaternary pump. Found phenolics and flavonoids.

Cell lines

VACSERA in Giza, Egypt, graciously provided colon cancer-associated Caco-2 cells. At Roswell Park Memorial Institute (RPMI 1640), cells received 5% heat-treated bovine serum albumin (BSA), 4 mM L-glutamine, and 4 mM sodium pyruvate. Cells were grown in a 75-milliliter flask at 37 degrees Celsius with 5% CO_2 [34, 35]. The Zeiss A-Plan 10X inverted microscope imaged cultured cells.

Proliferation assay

Cell morphology was examined using an inverted microscope. Cells were planted in duplicate at a density of 10×10^4 cells per well on a 6-well plate to promote proliferation. Hemocytometer measurements determined cell survival. Thus, the outdated medium was removed, the cells were trypsinized, and incubated at 37°C for three minutes after two PBS washes. After giving the trypsinized cells an adequate volume of complete RPMI media, cell morphology was studied under an inverted microscope.

Cytotoxic concentration 50% (CC50)

The extract's CC50 was estimated using Caco-2 cells after cytotoxicity testing. Cells were cultivated in 96-well plates at 10×10^3 cells per well in a CO_2 incubator at 37°C. Before overnight incubation, cells were treated with 0.3-5 mg/ml of each indicated extract. MTT cell growth test kit (Sigma-Aldrich, Germany) measured cell viability and cytotoxicity using formazan dye absorbance at 570 nm.

Lactate dehydrogenase (LDH) production

We used the LDH assay kit Abc-65393 to measure LDH production in cells exposed to 600 g/ml etch extract in their growth conditions. 100 l of lysed cells were treated with 100 l of LDH reaction mix at room temperature for 30 min. A plate reader assessed LDH activity at OD450 nm. The fold change from splitting the mean values of treated cells by the false values was used to compute relative LDH production [36, 37].

Enzyme-linked immunosorbent assay (ELISA)

Human ELISA kits (Abcam 100572 and 100575) measured released IL-6 and IL-8. The experiment incubated Caco-2 cells overnight in 96-well plates. The cells were treated with 600 µg/ml of each extract and incubated for 0, 6, 12, 24, 36, 48, and 72 hours. At every time point, 1X Invitrogen cell lysis solution from the U.S. destroyed the cells. After that, 50 l of 1X biotinylated antibody and 100 l of control solution were added to 100 l of lysed cells and incubated in the ELISA plate reader for 2 hours at room temperature. Then, each sample received 100 l of 1X streptavidin-HRP solution and was incubated for 30 minutes in a dark environment. Then, 100 L of chromogen TMB substrate was added to each sample well. Following this, the samples were incubated at room temperature for 15 minutes without light. Each sample received 100 L of stop solution to end the procedure. Each well's 450 nm absorbance was measured [38, 39].

Annexin V protocol

In a cell population with varied extract concentrations, the Annexin V-FITC Early Apoptosis Detection Kit (Cell Singling Technology, USA) identified early apoptotic cells. Phosphatidylserine expression was quantified, an indication of early apoptosis. In this investigation, cells were stained with propidium iodide (P.I.), a non-cell-permeable DNA dye. Necrosis cells measured 594nm. We collected treatment cells by centrifuging them at 1500 rpm for 3 minutes and washing them in ice-cold PBS. Resuspended cells had 96 L 1x Annexin V Binding Buffer, 1 L FITC Conjugate, and 12.5 L P.I. After being exposed to the solution, each sample was incubated on ice for 10 minutes under light. The stained cells received 250 L of 1X Annexin V Binding Buffer, ice-cold. The cells were analyzed with BD Accuri 6 Plus flow cytometry after labeling.

Quantitative Real-time PCR (qRT-PCR)

Gene expression was measured using qRT-PCR. The Invitrogen (USA) RNA purification kit and TriZol were used to get cellular total RNA. M-MLV reverse transcriptase from Promega in the U.S. synthesized cDNA from one gram of total RNA. Beclin 1, Atg5, PTEN, and Casp3 expression were evaluated using the QuantiTect-SYBR-Green PCR Kit (Qiagen, USA) and Table 1 primers. The housekeeping gene GAPDH mRNA expression level was utilized to analyze real-time PCR data. SYBR green (10 L), RNase inhibitor (25 units per L), 0.2 molar concentration of each primer, 2 L of produced cDNA, and nuclease-free water (25 L) made up the PCR reaction mixture. PCR began with 5 minutes of denaturation at 94 °C, followed by 35 cycles of 30 seconds of denaturation, 15 seconds of annealing at 60°C, and 30 seconds of extension at 72 °C [37, 40, 41].

Data analysis

Each histogram and graphic was methodically created in Microsoft Excel. Quantification of mRNA from qRT-PCR assay used Δ - Δ Ct analysis, based on equations: (1) Δ -Ct = Ct value for gene- Ct value for GAPDH, (2) (Δ – Δ Ct) = Δ Ct value for experimental – Δ Ct for control), (3) Quantification fold change = (2-Δ-Δ Ct) (1,2). The statistical analysis utilized by the student was the two-tailed t-test. It was deemed reasonable to use a P-value of less than or equal to 0.05 to evaluate statistical significance.

Results

Ginger, garlic, chamomile, and cinnamon quantitative analysis by analytical HPLC

Tables (2, 3, 4, and 5) show the ethanol extract flavonoid and phenolic changes for cinnamon, chamomile, ginger, and garlic. All extracts contained chlorogenic, catechol, and p-coumaric. Two extracts contained isoferulic, protocatechulic, and isoferulic (ginger and garlic). The flavonoids rutin, querestin, kampferol, and luteolin are also present. Garlic contains just coumaric and epicatechin.

Ginger and garlic have the most chlorogenic and protocatechulic substances at 6.3-6.8 and 11.26 and 14.66 g/mL, respectively. Catechin and p-coumaric are 35.16 and 17.36 g/mL in cinnamon, respectively.

GC-MS analysis

GC-MS identified bioactive components in ginger, garlic, chamomile, and cinnamon ethanol extracts. Parts are included in Supplementary Tables 6, 7, 8 and 9. Diallyl disulfide (32.19%), trisulfide di-2-propenyl (20.17%), 1-isopropenyltricyclohexane (8.62%), and ethyl aminomethyl formamide (5.74%) had the greatest peak areas in garlic extracts. At varying retention times, less than 5% of other compounds are present.

The largest peak area (%) components in ginger are zingiberene, α-bisabolene, and α-sesquiphellandrene (52.79, 12.57, and 20.15, respectively), while chamomile has α-farnesene (29.5) and bisabolol oxide (34.75). Alpha-copaene (42.00%), naphthalene (16.55%), cinnamaldehyde (E) (13.37%), à-muurolene (10.74%), and dimethylacetal (8.31%) were the strongest peak regions in cinnamon extracts.

Garlic and ginger, garlic, chamomile, and cinnamon extract significantly inhibit colon cancer cell proliferation.

The cytotoxicity of the four plant extracts (ginger, garlic, chamomile, and cinnamon) was The MTT test

Table 1. The Sequences of Oligonucleotides Utilized for the Quantification of mRNA Expression Levels of the Specified Genes.

Description	Primer sequences 5'-3'
Beclin-1-sense	ACCGTGTCACCATCCAGGAA
Beclin-1 antisense	GAAGCTGTTGGCACTTTCTGT
Atg5-sense	CGTGTATGAAAGAAGCTGATGC
Atg5-antisense	ACGAAATCCATTTTCTTCTGGA
PETN-sense	AAGACATTATGACACCGCCAAAT
PETN-antisense	ATGATTGTCATCTTCACTTAGCCATT
Casp3-sense	GGACAGCAGTTACAAAATGGATTA
Casp3-antisense	CGGCAGGCCTGAATGATGAAG
GAPDH-sense	TGGCATTGTGGAAGGGCTCA
GAPDH-antisense	TGGATGCAGGGATGATGTTCT

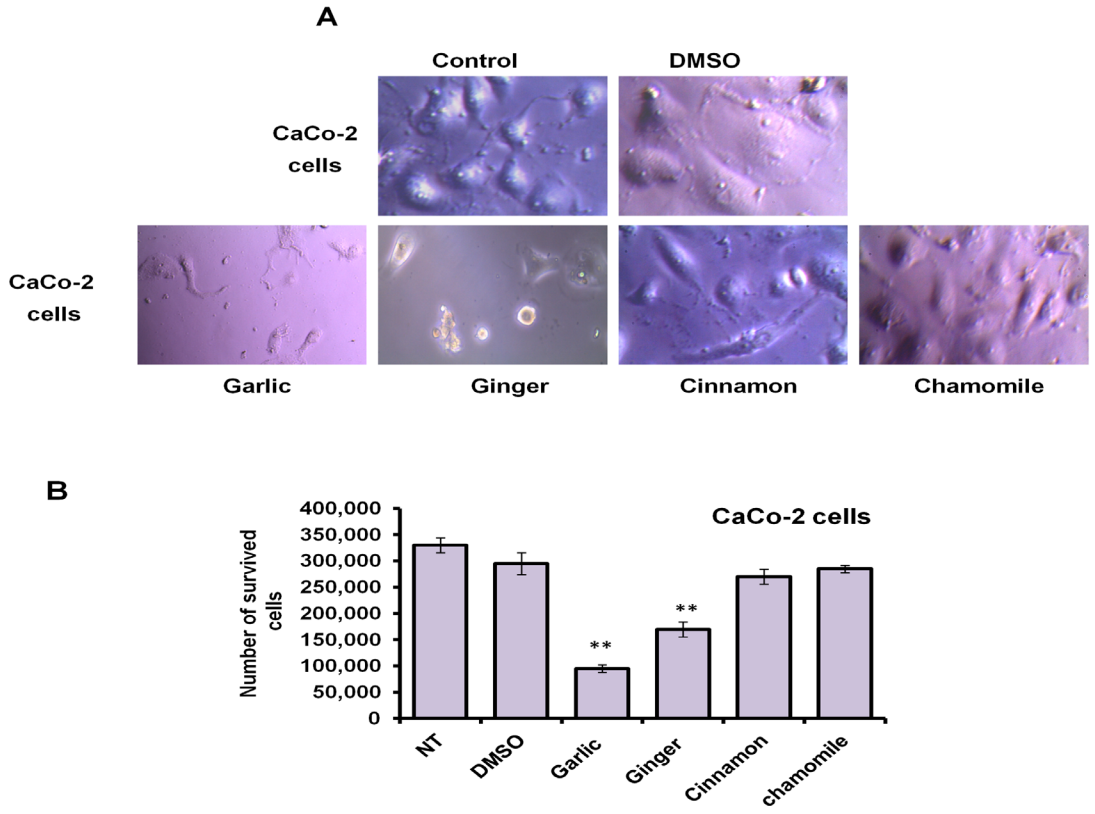

Figure 1. Cytotoxic Effect of the Specified Plant Extracts on Caco-2 Cells. (A) The quantity of survived cells that were subjected to treatments involving extracts derived from ginger, garlic, chamomile, and cinnamon. This study's error bars show four different studies' S.D.s. A student's two-tailed t-test was employed to analyze the values represented. The significance threshold is typically set at *p ≤ 0.05 and **p ≤ 0.01. (B) Representative inverted microscope photos depicting the cellular shape of cells two days after being treated with extracts of ginger, garlic, chamomile, and cinnamon, as well as cells treated with DMSO and untreated cells (N.T.).

was performed on Caco-2 human colon cancer cells after treatment with different concentrations of each extract (0 5 mg/mL). Caco-2 cells were treated with the plant extract overnight at 50% (CC50). The MTT experiment determined cell viability. Figure 1A shows that chamomile and cinnamon are somewhat cytotoxic in treated cells. Cell shape is affected by extract plants in Figure (1A). Cells without extracts (Control) have intact membranes. Garlic and ginger extracts caused apoptotic blebbing, but chamomile and cinnamon had a modest effect. Microscopic images (Figure 1B) showed that garlic and ginger reduced the number of live cancer cells compared

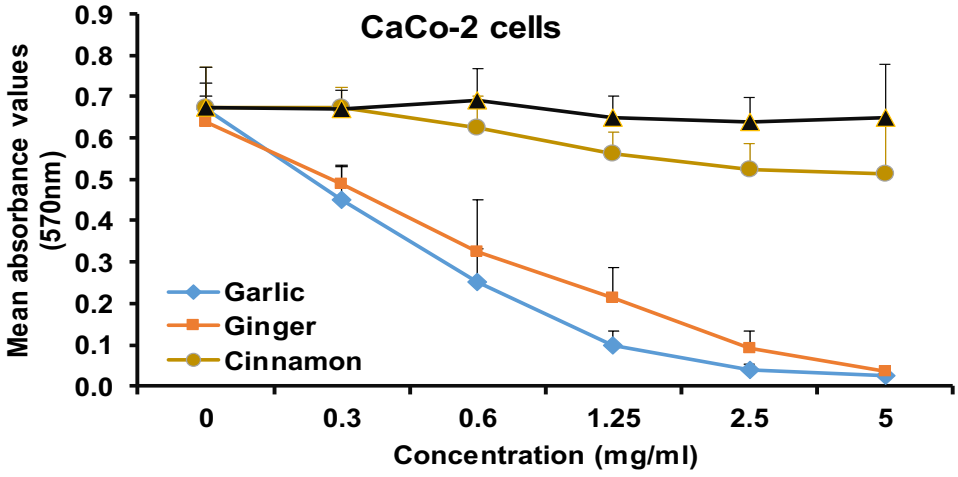

Figure 2. Potential Cytotoxic Effects of Indicated Plant Extraction in Caco-2 Cells. Cell viability rate of Caco-2 cell line in response to the indicated concentration of plant extortions upon 24 h post-treatment. The error bars show the four different replicates' standard deviations. The significance of the indicated values was investigated using the student two-tailed test.

Table 2. Results of a Specific Retention Time (R.T.)-based HPLC Test for Phenolic and Flavonoid Components in an Extraction of Garlic (Allium sativum)

Phenolic Compounds	Formula	R.T. (min)	Concentration (µg/ml)	Flavonoid Compounds	Formula	R.T. (min)	Concentration (µg/ml)
Syringic	$C_9H_{10}O_5$	3	3.55	Myrecetin	$C_{15}H_{10}O_8$	3	5.21
Gallic	$C_7H_6O_5$	4	2.14	Rutin	$C_{27}H_{30}O_{16}$	4.6	4.88
Protocatechulic	$C_7H_6O_4$	6.3	6.88	Naringine	$C_{27}H_{32}O_{14}$	5.2	3.48
Chlorogenic	$C_{16}H_{18}O_9$	8.1	11.26	Querestin	$C_{15}H_{10}O_7$	7	14.36
Catechal	$C_{15}H_{14}O_6$	9.3	6.19	Kampferol	$C_{15}H_{10}O_6$	8	1.79
Isoferulic	$C_{10}H_{10}O_4$	11	5.69	Luteolin	$C_{15}H_{10}O_7$	9.1	2.41
P-coumaric	$C_9H_8O_3$	13	4.36	Apigenin	$C_{15}H_{10}O_5$	10	1.98
Vanillic	$C_8H_8O_4$	15.6	5.99	Hyperoside	$C_{21}H_{20}O_{12}$	11	1.69
				Catechin	$C_{15}H_{14}O_6$	12	3.14
				Coumaric	$C_9H_8O_3$	16	5.69
				Epicatechin	$C_{15}H_{14}O_6$	18	9.73

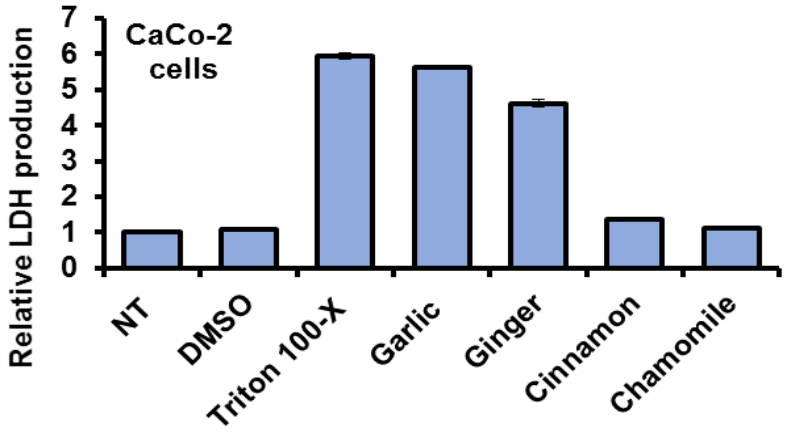

Figure 3. Relative LDH Production from Treated Cells Compared with Triton 100-X Treated Cells. Error bars indicate the standard deviation (S.D.) of four different replicates. The student's two-tailed test was used to determine P values and the significance of LDH production level.

to the control group treated with DMSO and other extracts.

As indicated in Figure 2, garlic and ginger extraction concentrations decreased cell death. Ginger and garlic inhibited 50% of Caco-2 cells at 1.25 mg/mL and 0.6 mg/mL, respectively. Supplementary Table 10 shows the T-TEST data analysis with a P 0.05 significance threshold.

LDH production in the treated cells

LDH enters culture media after cell membrane disintegration. Thus, LDH is a wide indication of cell viability and necrotic cell death. Figure 3 shows that garlic and ginger extracts increased relative LDH synthesis by 6 and 4 times, respectively, compared to chamomile and cinnamon extracts. Garlic and ginger extract affected Caco-2 cells better than triton 100-x. Thus, colon cancer LDH leakage is a cytotoxicity sign. Morphological alterations indicate cell death induced by garlic and ginger extraction, and the LDH assay in Supplementary Table 11

Table 3. Results of an HPLC Analysis of Phenolic and Flavonoid Components in an Extraction of Ginger (Zingiber Officinale)

Phenolic Compounds	Formula	R.T. (min)	Concentration (µg/ml)	Flavonoid Compounds	Formula	R.T. (min)	Concentration (µg/ml)
Pyrogallol	$C_6H_3(OH)_3$	4.98	8.12	Myrecetin	$C_{15}H_{10}O_8$	3	8.21
Protocatechulic	$C_7H_6O_4$	6.01	6.31	Rutin	$C_{27}H_{30}O_{16}$	4.5	3.17
Chlorogenic	$C_{16}H_{18}O_9$	8.01	14.66	Querestin	$C_{15}H_{10}O_7$	7	11.14
Catechol	$C_{15}H_{14}O_6$	9.02	4.87	Kampferol	$C_{15}H_{10}O_6$	8.2	8.36
Isoferulic	$C_{10}H_{10}O_4$	11	12.64	Luteolin	$C_{15}H_{10}O_7$	9.5	3.15
Ferulic	$C_{10}H_{10}O_4$	12	6.44				
P-coumaric	$C_9H_8O_3$	13	11.23				

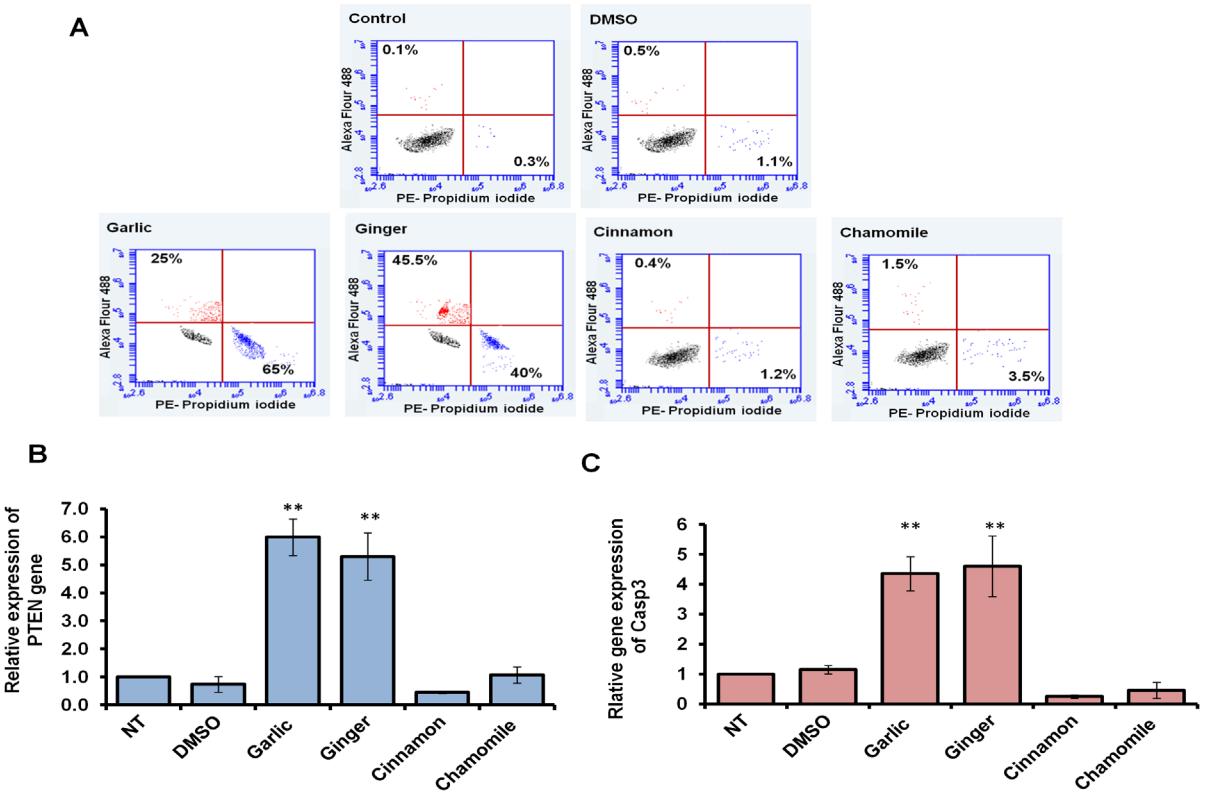

Figure 4. Cytotoxic Effect of the Specified Plant Extracts on Caco-2 Cells. (A) The quantity of survived cells that were subjected to treatments involving extracts derived from ginger, garlic, chamomile, and cinnamon. This study's error bars show four different studies' S.D.s. A student's two-tailed t-test was employed to analyze the values represented. the significance threshold is typically set at *p ≤ 0.05 and **p ≤ 0.01. (B) Representative inverted microscope photos depicting the cellular shape of cells two days after being treated with extracts of ginger, garlic, chamomile, and cinnamon, as well as cells treated with DMSO and untreated cells (N.T.).

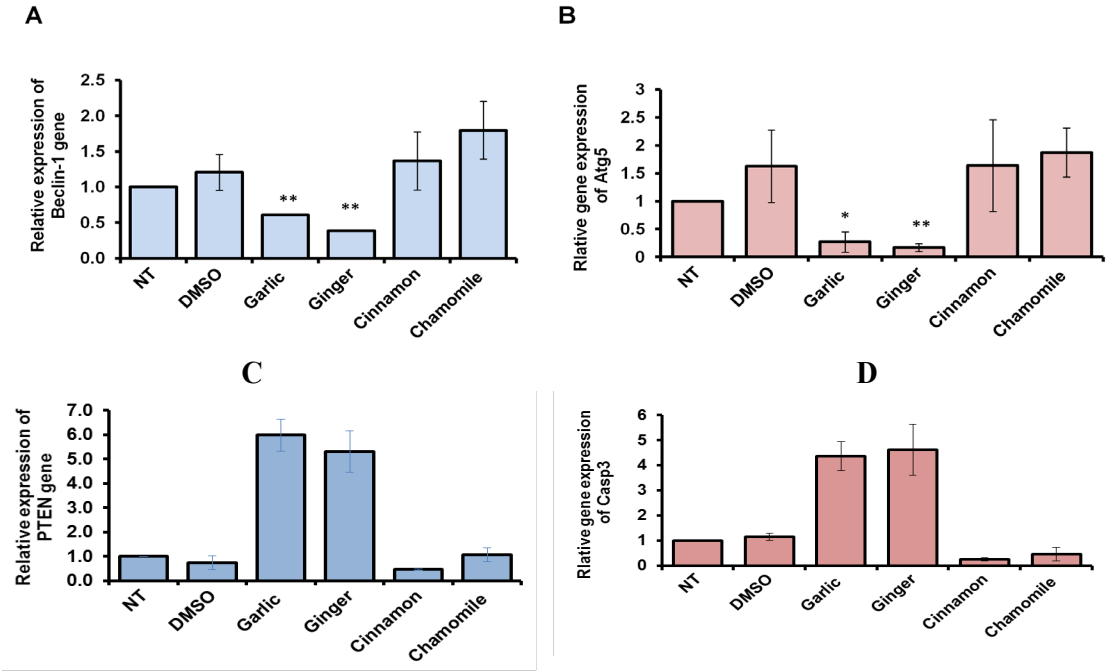

Figure 5. Relative Expression of Beclin1, PTEN, Atg5, and Caspase-3 in Treated Caco-2 Cells. (A) Steady-state mRNA of Beclin1 gene indicated by fold change in Caco-2-treated cells compared with DMSO-treated cells. (B) Steady-state mRNA of PTEN gene indicated by fold change in PC3 cells in comparison with DMSO-treated cells. (C) Steady-state mRNA of Atg5 gene indicated by fold change in Caco-2 cells in comparison with DMSO-treated cells. (D) Steady-state mRNA of Atg5 gene indicated by fold change in Caco-2 cells in comparison with DMSO-treated cells. Levels of GAPDH-mRNA were used as an internal control. The SD of three independent experiments is shown by error bars. Cycle threshold (Ct) significance analysis employed the student's two-tailed t-test. *p ≤ 0.05, **p ≤ 0.01.

Table 4. Results of an HPLC Analysis of Chamomile (Matricaria chamomilla) Extraction for Phenolic and Flavonoid Compounds

Phenolic Compounds	Formula	R.T. (min)	Concentration (µg/ml)	Flavonoid Compounds	Formula	R.T. (min)	Concentration (µg/ml)
Syringenic	$C_9H_{10}O_5$	3.1	3.66	Myrecetin	$C_{15}H_{10}O_8$	3	3.11
Gallic	$C_7H_6O_5$	4	5.14	Rutin	$C_{27}H_{30}O_{16}$	4.3	2.98
Chlorogenic	$C_{16}H_{18}O_9$	8.3	0.69	Querecetin	$C_{15}H_{10}O_7$	7.2	15.36
Catechol	$C_{15}H_{14}O_6$	8.95	6.31	kampferol	$C_{15}H_{10}O_6$	8	4.79
P -O.H. benzoic	$C_7H_6O_3$	10	13.47	Luteolin	$C_{15}H_{10}O_7$	9.5	6.77
Caffeic	$C_9H_8O_4$	11.2	11.22	catechin	$C_{15}H_{14}O_6$	12	16.09
Ferulic	$C_{10}H_{10}O_4$	12.1	8.06				
P -coumaric	$C_9H_8O_3$	13	9.43				

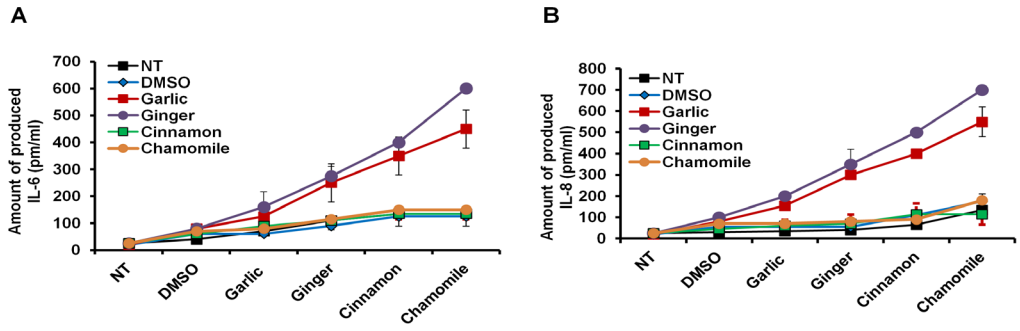

Figure 6. Levels of pro-Inflammatory Cytokines IL-6 and IL-8 in Cells that have been Treated. (A) The amount of generated IL-6 (pm/mL) in the fluids media of Caco-2 cells that received treatment with DMSO versus untreated cells at the indicated time points. (B) The amount of IL-8 generated by infected cells subjected to the plant extract, as measured in pm/mL.

shows necrosis was the main cause.

The level of phosphatidylserine (PTEN and Casp3) expression

PTEN and Casp3 were detected using a flow cytometry assay.

Cancer is characterized by its ability to evade apoptosis. Since the cell community is well-controlled, normal cells die by apoptosis when no longer needed. PTEN and Caspase-3 activity was measured after treating Caco-2 cells with mmol/min/ml ginger, garlic, chamomile, and cinnamon (based on LDH cytotoxicity).

Figure 4 shows ginger-garlic-treated cells. All garlic and ginger extract doses increased PTEN and Caspase-3. Garlic and ginger raised PTEN and Caspase-3 activity in Caco-2 cells to 1.2 to 2.35 mmol/min/ml, while chamomile and cinnamon extracts had a dose effect (4.5 mmol/min/ml) (Figure 4).

Gene expression determination

In Caco-2 cells treated with natural plant extracts, Beclin1, PTEN, Atg5, and Caspase-3 mRNA levels were measured to examine their effects on cellular signaling. Quantification was done with qRT-PCR. Caco-2 cells received ginger, garlic, chamomile, and cinnamon. The cells' total RNA and c-DNA were then retrieved and showed Beclin1 inhibition by garlic and ginger at 0.6 mg/mL and 0.39 mg/mL, respectively. These impacts were significant (P = 0.0001). Cinnamon and chamomile also increased Beclin1 expression by more than 3 and 5 fold, respectively, but these changes were not statistically

Table 5. Results of an HPLC Analysis for Phenolic and Flavonoid Components in the Extraction of Cinnamon (Cinnamomum cassia)

Phenolic Compounds	Formula	R.T. (min)	Concentration (µg/ml)	Flavonoid Compounds	Formula	R.T. (min)	Concentration (µg/ml)
Pyrogallol	$C_6H_3(OH)_3$	5	10.33	Rutin	$C_{27}H_{30}O_{16}$	4.4	9.14
Catechin	$C_{15}H_{14}O_6$	7	35.16	Naringine		5.2	8.16
Chlorogenic	$C_{16}H_{18}O_9$	8	6.47	Querestin	$C_{15}H_{10}O_7$	7	15.23
p-PH benzoic	$C_7H_6O_3$	9.8	8.16	Kampferol	$C_{15}H_{10}O_6$	7.9	6.17
Ferulic	$C_{10}H_{10}O_4$	12	9.12	Luteolin	$C_{15}H_{10}O_7$	9.1	7.46
P-coumaric	$C_9H_8O_3$	13	17.36	Apigenin	$C_{15}H_{10}O_5$	10	14.56
Vanillic	$C_8H_8O_4$	15.6	8.66	Catechin	$C_{15}H_{14}O_6$	12	9.52

significant (P = 0.330 and P = 0.108).

To investigate the hypothesis that plant extract-induced Autophagy causes cell death. Garlic and ginger were tested for PTEN effects (Figure 5C, D). PTEN and caspase were upregulated more than 6 and 5 fold, respectively. Supplementary Table (12a-d) shows that garlic and ginger decreased PTEN and caspase3 expression in Caco-2 more than in cinnamon and chamomile.

ELISA-based quantification of IL-6 and IL-8 secretion

To measure pro-inflammatory cytokines from interleukin-expressing human tumor cells treated with ginger, garlic, cinnamon, and chamomile. After 24h, IL-6 and IL-8 concentrations increased significantly compared to untreated or DMSO-treated cells. Instead, cinnamon and chamomile extract reduced IL-6 and IL-8 (Figure 6).

Discussion

Ethnic groups worldwide have used phytomedicines to treat and prevent ailments for centuries. Traditional or herbal medicines are often used in 3rd world countries for basic health care due to their lower side effects, affordability, and biocompatibility. In traditional cultures, herbal treatments are easily available and preferred as therapeutic agents. About 25% of U.S. medications incorporate plant-based components. Plant-based active medicinal ingredients and phytochemicals with anticancer, antioxidant, anti-inflammatory, and proapoptotic properties are scarce in the pharmaceutical industry [42]. Oncology uses several plant-derived substances for prevention and treatment. Many more plant extracts are in clinical trials at various stages [43].

Ginger, garlic, chamomile, and cinnamon included phenolic, flavonoid, and sulfur components in HPLC and GCM tests. Ginger may prevent cancer due to flavonoids and polyphenolics, particularly quercetin [44]. LC-MS/MS analysis validated the key chemicals in Kayiran et al.'s. [45] investigation. Our study found that hydrolysis can produce quinic acid from chlorogenic acid. Takim et al. and Qiu et al. found that garlic species had similar photochemical components but in different amounts [46].

The MTT assay was used to determine the cytotoxic concentration IC50 (0.6 and 1.25 mg/ml) of ginger and garlic extract in Caco-2 cells. According to Duaa et al. [47] and Bagul et al. [48], crude garlic extract reduced cell proliferation by 80% to 90% in Hep-G2, PC-3, and other cancer cell lines. Takim et al., [46], discovered that Allium tuncelianum (MAT) extracts were more cytotoxic to ECC-1 and PC3 cells. In this study, cinnamon and chamomile extract had moderate to modest effects. In contrast, cinnamon has been shown to be cytotoxic to HeLa, HL-60, HCT 116, H.T. 29, SW 480, A431, and SiHa [49]. Takim et al. found that the CC50 of MAT Turkish garlic was cytotoxic at 0.105 mg/mL in PC3 cells, compared to 1.25 mg/mL in the current study, depending on the garlic species. Ginger inhibits proliferation and promotes apoptosis in the murine melanoma B164A5 cell line, according to Danciu et al. [50]. However, ginger has a significant cytotoxic effect on Raji cells originating from human Burkitt's lymphoma [51]. Morphologically, ginger extract-treated cells showed a significant degree of cytoplasmic vacuolization 24 hours after treatment, with a consensus that these vacuoles are likely autophagosomes due to the GFP-LC3 tip.

LDH, a crucial enzyme in live cells, indicates cellular damage or harm when high. LDH release increased with garlic and ginger extracts at 0.3 to 5mg/ml. Chamomile and cinnamon extracts at 4.5mg to 5mg/ml showed only a slight increase in cytotoxicity. The production of LDH from Caco-2 cells strongly shows that garlic and ginger extracts may kill cancer cells. Another study found dose-dependent cytotoxic effects of Allium sativum (garlic) and its bioactive sulfur components [52-54]. The Caco-2 cells under examination are colon cancer cells, which can avoid apoptosis and survive. (2019, Willis et al.) Diallyl disulfide (DATS) in garlic extract inhibits cell cycle progression, tumor cell proliferation, and angiogenesis, fighting tumors [55, 56].

The 150-kilobase BECN1 gene on the 17q21 chromosome of Homo sapiens is similar to Saccharomyces cerevisiae's atg6/vps30 gene. Autophagy regulation by BECN1 contributes to carcinogenesis [57, 58]. The scientific community disagrees on whether Autophagy inhibits carcinogenesis or allows adaptive tumor responses [59]. Ginger and garlic extract reduced PTEN and caspase3 protein expression in Caco-2 cells compared to chamomile and cinnamon. Suggests that PTEN is involved in ginger and garlic autophagy in colon cancer cell lines. Caspases, cysteine-dependent proteases, are involved in apoptosis and other cellular processes [60-62]. By promoting apoptosis, caspase-3 activation reduces the risk of cancer [63]. 2001. Apoptosis is regulated by caspases, which activate DNAse to degrade DNA in the cell nucleus. The cell dies from phagocytosis [64].

To illustrate the biological effects of natural plant extracts on cellular signaling, qRT-PCR measured Beclin1, PTEN, Atg5, and Caspase-3 mRNA levels in treated Caco-2 cells. Garlic and ginger caused more cell death than cinnamon and chamomile extract when ATG5 or Beclin1, which are necessary for Autophagy, were knocked down. Garlic and ginger treatments increased PTEN and caspase3 expression genes by 6 and 5 times, respectively, in Caco-2 cells compared to cinnamon and chamomile. Szychowski et al. [65] found that only 0.062 mg/mL of extract from the Spanish garlic cultivar Morado increased caspase-3 activation. Su et al. [66] discovered caspase-3-dependent apoptosis in Colo 205 cells with garlic extract dosages of 0.0005–0.002 mg/mL. Kim et al. [67] found that garlic clove hexane extracts at 100 lg/mL (0.1 mg/mL) induced apoptosis in Hep3B cells. After 48 hours, reactive oxygen species and caspase-3, caspase-8, and caspase-9 activation induced this impact. Ginger and gingerol exhibit antiproliferative, anticancer, and anti-invasive properties in vitro, in vivo, and in clinical investigations. These approaches entail altering NF-κB, ERK, Akt, Rb, PI3K, MAPK, STAT3, cIAP1, cyclin A, Cdk, cathepsin D, and caspase-3/7 [68]. Ginger ingestion also slowed the cell cycle, lowered reproductive capability, and caused caspase-mediated mitochondrial death [69].

Ginger extract treatment in U251 and HCT116

cancer cells led to decreased cell viability, cytochrome c-mitochondrial release, increased Bax: Bcl2 ratio, activated caspase-3 and -9, and cleaved PARP [70]. Ginger extract inhibited many antiapoptotic proteins, including nuclear NF-κBp65, Mcl-1, BclX, survivin, Bcl2, and XIAP. Cyclin D1 and cyclin/Cdk-4 were downregulated, while p53, p21, and p27 were increased. Ginger extract increased p53 and p21 and decreased nuclear NF-Bp65, survivin, XIAP, and cyclin D1 [71]. Medicinal plants have long been used in alternative and supplementary medicine. Turmeric, ginger, and garlic extracts demonstrate free radical scavenging and anticancer activity against ZR-75, MCF-7, and MDA-MB 231 [72].

Garlic and ginger therapy increased IL-6 and IL-8 levels after 24 hours compared to untreated and DMSO-treated cells. Cinnamon and chamomile extract reduced IL-6 and IL-8—garlic and ginger extract control pro-inflammatory cytokines. Saryono et al. [73] found that black garlic extracts reduce IL-6 and IL-8 expression. When cells engage the autophagy gene signaling system, they create more pro-inflammatory cytokines, including IL-6 and IL-8. IL-6 and IL-8 synthesis maintained cellular proliferation, viability, inflammatory processes, and carcinogenic events. 2021 (El-Fadi et al.) After an oncogenic event, interleukin signaling in neoplastic cells may promote tumor development, metastasis, and cancer progression [74].

In conclusion, this study examined if Egyptian plant extracts (ginger, garlic, cinnamon, and chamomile) in DMSO may treat cancer. Analyses and studies showed 1.25 mg/mL garlic and ginger cytotoxic to Caco-2. In Caco-2 cells, garlic and ginger extract increased LDH synthesis five times more than cinnamon and chamomile extracts. Ginger and garlic components like PTEN and Caspase-3 affected proapoptotic signaling more than Beclin1 and Atg5.

Garlic and ginger extract activates the mitochondria-dependent caspase-3 pathway in Caco-2 cells, causing apoptosis, boosting PTEN expression, and down-regulating Beclin1 and Atg5 autophagy genes compared to cinnamon and chamomile. Garlic and ginger elevated IL-6 and IL-8 in treated cells higher than cinnamon and chamomile using ELISA at the given times. The results demonstrated garlic extract regulates pro-inflammatory cytokines.

Garlic and ginger-based herbal medicines are safe and effective anticancer treatments for Caco-2, which may interest public health authorities and pharmaceutical firms. Doctors, scientists, food technologists, and nutritionists need garlic and ginger supplements.

Author Contribution Statement

All authors contributed to the study idea and design, experimental work, paper writing, editing, and revision. Availability of data and materials

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Ethical approval and consent to participate

Not applicable.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The study authors affirm that no conflicts of interest could influence the findings.

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