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– ORIGINAL PAPER –

Differential Effects of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*) Extracts on Malignant and Normal Hematopoietic Cells in the Bone Marrow Microenvironment

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Abstract

Natural compounds are increasingly investigated for their potential as complementary therapies in hematologic malignancies. In this study, we examined ethanol extracts of turmeric (Curcuma longa) and ginger (Zingiber officinale) rhizomes, two phytocompounds with a long history of use in traditional medicine but limited evaluation as complex extracts in myeloid neoplasms. Phytochemical analysis revealed that turmeric extract contained a significantly higher concentration of total polyphenols compared to ginger, while GC–MS demonstrated distinct chemical profiles enriched in tumerones and curlone for turmeric and sesquiterpenes such as 7-epi-sesquithujene and β-sesquiphellandrene for ginger. Functional assays showed that both extracts reduced the viability of malignant myeloid blasts in a dose-dependent manner, with ginger demonstrating greater potency reflected by lower IC_{50} *values. Flow cytometry confirmed induction of apoptosis in leukemic cells, including in co-culture with mesenchymal stromal cells, suggesting the ability to overcome microenvironmental protection. At higher concentrations, both extracts also decreased the viability of normal* $CD34^+$ *progenitors, although the effects were less pronounced at lower doses, indicating a degree of selectivity compared to malignant myeloid cell. Taken together, these results highlight the distinct and context-dependent biological activities of turmeric and ginger extracts, their potential to disrupt stromal support for malignant myeloid cells, and their relative sparing of normal hematopoietic progenitors at lower concentrations. These findings provide a rationale for further preclinical exploration of standardized extracts as complementary strategies in myeloid malignancies.*

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Introduction

Leukemias are malignant diseases of the hematopoietic system characterized by the abnormal and progressive accumulation of precursor cells of the myeloid or lymphoid lineage (1). They can present in acute or chronic forms, with acute subtypes often behaving aggressively and remaining therapeutically challenging. Current treatment of acute myeloid leukemia (AML) relies largely on intensive chemotherapy, now complemented by targeted therapies such as FLT3 and IDH inhibitors or venetoclax-based combinations. Although these advances have improved remission rates and overall survival, relapse is frequent, most patients ultimately succumb to their disease, and many therapies are associated with substantial toxicity, limiting their tolerability, especially in older adults.

In this context, research into natural compounds of plant origin has gained renewed interest. Early studies with alkaloids such as vinca derivatives and with retinoids like all-trans retinoic acid demonstrated striking activity in certain leukemias, fueling optimism that was later tempered by limited efficacy in broader settings (2). More recently, however, a growing body of work has highlighted the potential of phytocompounds not only as therapeutic alternatives but also as adjuvants to conventional regimens. These advances raise hope for their role in primary prevention, or in secondary prevention strategies aimed at reducing relapse risk when combined with standard therapies.

Phytocompounds long employed in traditional Asian medicine have demonstrated diverse biological activities relevant to cancer prevention and therapy. Among them, turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) have attracted particular attention and are increasingly studied for their rich content of bioactive metabolites with therapeutic potential (3, 4, 5). A notable feature of these compounds is their low toxicity profile, as they are generally well tolerated even at relatively high doses. Furthermore, their widespread and long-standing use both as culinary spices and as phytopharmaceuticals provides a unique advantage, supporting their candidacy for roles in primary prevention or as adjuvant agents in modern oncological treatments.

While most studies have focused on purified active compounds—such as curcumin from turmeric and gingerol or shogaol from ginger—rather than complex plant extracts, this reductionist approach has been critical for elucidating the cellular and molecular mechanisms by which these phytocompounds exert their effects.

Curcumin has been shown to downregulate DNMT1, leading to reactivation of silenced tumor suppressor genes and apoptosis in AML cells (6), to suppress FLT3/STAT5 signaling and induce cell cycle arrest (7), and to inactivate AKT, thereby promoting G1 arrest and apoptosis (8). It can also trigger pyroptosis through inflammasome activation and gasdermin D cleavage, suggesting a novel mode of cell death in sensitive AML subtypes (9). In parallel, ginger-derived compounds such as 6-gingerol have been reported to induce apoptosis via ROS accumulation, DNA damage, and miR-27b upregulation, leading to NF-κB pathway suppression (10), and to modulate DNMT1/3A expression and tumor-suppressor miRNAs in NB4 cells (11). More recently, shogaol has been identified as particularly potent against leukemic stem-like cells, acting through Akt inhibition, JNK/c-Jun activation, and WT1/CD34 downregulation (12). Together, these findings provide mechanistic insight into how turmeric- and ginger-derived metabolites may complement conventional therapies in AML, yet much less is known about the broader impact and therapeutic potential of the complex plant extracts from which these compounds are derived.

In this study, we aimed to expand current knowledge by examining the effects of ginger and turmeric extracts, rather than isolated active compounds, on malignant myeloid cells. To this end, we employed models of both AML and myelodysplastic syndromes (MDS), the latter representing a clinically relevant precursor state. In parallel, we evaluated the impact of these complex extracts on normal and malignant hematopoietic progenitor cells, thereby assessing their selectivity and potential therapeutic window. Finally, because the bone marrow microenvironment critically shapes leukemic cell behavior, we also tested their activity in the context of bone marrow mesenchymal stromal cells, providing insight into how these phytocompounds may act under more physiologic conditions.

Materials and Methods

Preparation of ethanol extracts from plants

Fresh rhizomes of *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) obtained from certified organic crops were used for the preparation of ethanol extracts. After thorough cleaning to remove impurities, the rhizomes were cut into small pieces and subjected to maceration in 96% ethanol (Sigma-Aldrich, Germany) at a plant-to-solvent ratio of 1:2 (w/v).

Maceration was carried out under controlled light and temperature conditions for two weeks, with periodic shaking to maximize the extraction of bioactive phytocompounds. At the end of this period, the crude extracts were separated from the plant residue by gravity filtration using 150 mm diameter filter paper (Prat Dumas, France, Solantis), and the clear liquid phase was collected and stored for subsequent experiments.

The total phenolic content of each extract was determined using the Folin–Ciocalteu colorimetric method as previously described (13). Briefly, aliquots of the extracts were incubated with Folin–Ciocalteu reagent (Sigma-Aldrich, Germany) followed by the addition of sodium carbonate solution, and the mixtures were stored in the dark to allow color development. Absorbance was measured spectrophotometrically (Specord, Carl Zeiss, Germany) at 765 nm after two hours, and results were expressed as gallic acid equivalents (mg GAE/L) based on a standard calibration curve prepared with gallic acid.

Analysis of the chemical composition of the extracts

The chemical profile of the ethanol extracts of turmeric and ginger rhizomes was analyzed by gas chromatography–mass spectrometry (GC–MS) using a Clarus 500 system (Perkin Elmer, Shelton, CT, USA). Separation of volatile and semi-volatile components was achieved on a 60 m capillary column with an internal diameter of 0.32 mm, optimized for plant metabolite profiling. The injector temperature was maintained at 250 °C, and helium was employed as the carrier gas at a constant flow rate of 1 mL/min.

The GC oven program was set to an initial temperature of 100°C, which was increased at a rate of 4°C/min to a final temperature of 280 °C. Mass spectra were recorded in electron impact mode and compared with reference spectra in the instrument's database (NIST/EPA/NIH Mass Spectral Library) for compound identification. This approach allowed characterization of the principal phytoconstituents present in the turmeric and ginger extracts, including curcuminoids, terpenoids, and gingerols, in line with previously described methods for rhizome extract analysis (4, 13).

In the GC-MS determinations, concentrations were calculated by normalization, and the total polyphenol content in each solution was expressed as mg GAE/mL. Consequently, in all subsequent analyses, the polyphenol concentration in the system was reported as (µg GAE/mL).

Cell culture

<u>Cell lines:</u>

The MV4-11 AML cell line (ATCC, Manassas, VA, USA) was maintained in R10 complete medium consisting of RPMI-1640 (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), 2 mM L-glutamine (Life Technologies, Carlsbad, CA, USA), and 100 µg/mL penicillin/streptomycin (Gibco). The myelodysplastic syndrome–derived MDS-L cell line was cultured in R10 medium further supplemented with 10 ng/mL recombinant human interleukin-3 (IL-3; GeminiBio, Sacramento, CA, USA). Both cell lines were maintained in the exponential growth phase by routine splitting twice weekly and cultured in 25 cm^2 tissue culture flasks (Corning) at 37°C in a humidified atmosphere containing 5% CO_2.

<u>Primary cells:</u>

Normal bone marrow cells were obtained by washing collection filters from healthy donors undergoing bone marrow harvest for allogeneic transplantation at Johns Hopkins Hospital. All donors provided informed consent under a research collection protocol approved by the Johns Hopkins Institutional Review Board and in accordance with the Declaration of Helsinki. Mononuclear cells were processed to isolate $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) using magnetic bead–based selection (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions, as previously described (14). Normal mesenchymal stromal cells (MSCs) were generated from healthy bone marrow following previously published methods (14), cryopreserved at passage 1, and used in experiments at passages fewer than 4.

Experimental Design and Treatment Conditions to Assess Viability

Cell viability was evaluated using either a luminescence-based assay and by counting with Trypan blue.

<u>For luminescence-based assay:</u>

MV4-11 and MDS-L cells were seeded at 6,000 cells/well, and normal CD34$^+$ HSPCs at 3,000 cells/well, in 96-well plates. Cells were treated with either vehicle control (ethanol) or ethanol extracts prepared from fresh turmeric and ginger rhizomes. Extract concentrations ranged from 6.25 to 0.006 µg GAE/L (serial twofold dilutions: 6.25, 3.125, 1.562, 0.781, 0.39, 0.195, 0.097, 0.048, 0.024, 0.012, and 0.006 µg GAE/L).

Cultures were maintained for 72 hours at 37 °C in a humidified incubator with 5% CO_2.

At the end of the incubation period, 20 µL of CellTiter-Glo® reagent (Promega, Madison, WI, USA) was added to each well according to the manufacturer's instructions. Plates were incubated for an additional 24 hours to allow luminescence development, and absorbance was recorded using a CLARIOstar® plate reader (BMG Labtech, Ortenberg, Germany).

<u>For Trypan Blue Counting:</u>

Cells were seeded in 24-well plates at densities of 25,000 cells/well for MDS-L and MV4-11, and 20,000 cells/well for CD34$^+$ HSPCs. Cultures were treated with either vehicle control or ethanol extracts prepared from turmeric and ginger rhizomes at concentrations of 6, 3, 1.5, 0.75, 0.375, and 0 µg GAE/L, and incubated for 72 hours at 37 °C in a humidified atmosphere containing 5% CO_2.

Following treatment, cells were harvested by gentle but thorough pipetting, mixed with 0.4% Trypan Blue solution (Gibco, Thermo Fisher Scientific), and counted manually using a Neubauer hemocytometer under an optical microscope. Only unstained (viable) cells were recorded. This analysis was performed for both monocultures and co-cultures with MSCs to account for the potential protective effects of the bone marrow stromal compartment.

Flow cytometry analysis

Flow cytometry was used to evaluate cell viability following treatment with ethanol extracts. Analyses were performed on normal CD34$^+$ HSPCs, MDS-L or MV4-11 cells either in suspension or in co-cultures with MSCs. For co-culture experiments, MSCs were seeded in 24-well plates and grown to confluence. Subsequently, 25,000 MDS-L or MV4-11 cells/well were added on top of the MSC monolayer. Cells were treated with either vehicle control (ethanol) or ethanol extracts of turmeric rhizomes at concentrations of 6, 3, 1.5, 0.75, 0.375, and 0 µg GAE/L, and incubated for 72 hours at 37 °C in a humidified atmosphere with 5% CO_2.

After treatment, cells were collected by vigorous pipetting. An initial viability assessment was performed by Trypan Blue exclusion, and a portion of each suspension was then processed for flow cytometry. Cells were centrifuged (5 min at 300 g), resuspended in buffer containing PBS (Gibco), 3 µg/mL 7-aminoactinomycin D (7-AAD, Sigma), and 3 µg/mL Po-Pro-1 (Sigma), and 400 µL of this suspension was used per sample. Flow cytometric analysis allowed distinction of viable cells, apoptotic cells (Po-Pro-1$^+$/7-AAD$^-$), and dead cells (7-AAD$^+$).

For CD34$^+$ cells, parallel treatments with vehicle control, turmeric, or ginger extracts were performed, followed by sequential assessment with Trypan Blue exclusion and flow cytometry using the same staining protocol.

Statistical analysis

All experiments were performed in triplicate for each experimental condition. Data are presented as mean ± standard deviation (SD). Half-maximal inhibitory concentration (IC_{50}) values were calculated by nonlinear regression using the log(inhibitor) vs. response – variable slope model implemented in GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Comparative analyses between treatment conditions were based on IC_{50} values and variation across replicates. A p-value < 0.05 was considered statistically significant.

Results

Total Polyphenol Content and Volatile Compound Analysis of Turmeric and Ginger Extracts

The total polyphenol content, expressed as mg GAE/L, was higher in turmeric extract (1980 ± 37.62 mg GAE/L) compared to ginger extract (91 ± 1.82 mg GAE/L). This pronounced difference highlights the distinct chemical composition of the two plants and suggests potential differences in their biological and cytotoxic activities.

Gas chromatography–mass spectrometry (GC–MS) analysis further revealed a wide range of bioactive compounds in both extracts (Table 1). In turmeric, the predominant constituents were Tumerone (35.43%), Curlone (19.90%), and AR-tumerone (16.39%), all of which are well recognized for their diverse biological activities, including antitumor effects. By contrast, the ginger extract was characterized primarily by 7-epi-Sesquithujene (25.41%), β-Sesquiphellandrene (12.28%), and α-Curcumene (10.15%), compounds that contribute to its unique phytochemical and functional profile.

For most compounds, the probability of mass spectral match exceeded 95%, providing high confidence in their identification against the NIST and NBS libraries.

Plant	Polyphenols content (mg GAE/L ±STDEV)	Compound name	TR* (min)	P* (%)	Conc. (%)
Curcuma longa (turmeric)	1980±37.62	Alpha-phellandrene	9,32	98,3	1,55
		1-methyl-3-(1-methylethyl)-benzene (m-Cimen)	9,53	97,3	0,39
		D-Limonene	9,59	97,1	0,19
		Eucalyptol	9,67	99,0	2,70
		Gamma,-terpinene	9,91	95,9	0,14
		1-Methyl-4-(1-methylethylidene)-cyclohexene (Terpinolen)	10,24	98,4	0,43
		L-,Alpha,-terpineol	11,44	96,8	0,20
		2,3-Dihidro-benzofuran	11,73	92,4	0,90
		2-Methoxy-4-vinylphenol	12,58	95,0	1,81
		[1R-(1R*,4Z,9S*)]-4,11,11-trimethyl-8-methylene-Bicyclo[7.2.0]undec-4-ene(Caryophyllene)	13,67	95,6	0,36
		1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene (α-Curcumene)	14,03	98,7	1,33
		[S-(R*,S*)]-5-(1,5-dimethyl-4-hexenyl)2-methyl-1,4-cyclohexadine (α-Zingiberene)	14,14	97,2	1,99
		1,1'-(1,1,2,2-Tetramethyl-1,2-ethanedyl)bis-benzene (Dicumene)	14,21	98,8	0,09
		Beta-bisabolene	14,25	96,5	0,53
		3-(1,5-Dimethyl-4-hexenyl)-6-methylene-[S-(R*, S*)]-cyclohexene (beta.-Sesquiphellandrene)	14,41	98,1	2,90
		Bis(2-ethylhexyl)ester, hexanedioic acid	14,45	94,8	1,57
		2-Methyl-6-(P-tolyl)hept-2-en-4-ol (ar-Turmerol)	14,83	93,8	0,69
		(1R, 4R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohex-2-enol (Zingiberenol)	15,14	88,8	0,22
		AR-Tumerone	15,52	97,9	16,39
		Tumerone	15,57	98,5	35,43
		Curlone	15,84	97,7	19,90
		(6R, 7R)-Bisabolone	16,16	95,9	0,53
		(E)-Atlantone	16,40	95,3	1,24
		Dioctyl ester, hexanedioic acid	16,96	98,1	2,39
		6-(2-Hydroxy-4-methylphenyl)-2-methylhept-2-en-4-one (Tumeronol B)	17,29	92,7	0,33
		(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone ((6S,7R)-Bisabolone)	17,50	95,2	0,55
		Ethyl (E)-ferulate	17,60	97,3	0,14
		Butyl octyl ester 1,2-benzenedicarboxylic acid (Phthalic acid, butyl octyl ester)	17,77	95,1	1,32
		Dioctyl ester, hexanedioic acid (izomer)	19,31	98,2	0,91

		Methyl ester 9, 12-octadecadienoic acid (Z,Z) (Linoleic acid, methy; ester)	19,96	95,8	0,28
		Dioctyl ester, hexanedioic acid (izomer)	21,62	98,5	0,88
		Dioctyl ester, hexanedioic acid (izomer)	23,84	98,7	1,71
Zingiber officinale (ginger)	91±1.82	Hexanal	6,66	98,2	3,04
		Alpha-pinene	8,47	98,9	1,22
		(-)-Camphene	8,71	98,4	3,67
		Beta-myrcene	9,05	97,5	0,82
		D-Limonene	9,61	96,7	0,97
		Beta-phellandrene	9,66	97,5	2,55
		Eucalyptol	9,69	98,8	3,39
		Endo-borneol	11,28	97,3	1,34
		Dodecane	11.33	96,0	0,71
		Decanal	11,42	91,3	2,00
		2,6-Octadienal, 3,7-dimethyl-(E)- (α-Citral)	12,08	92,0	0,92
		Tetradecane	13,21	95,9	0,56
		Copaene	13,25	94,7	0,57
		6-Dodecen- 1-ol	13,46	94,4	0,95
		(E)-Beta-famesene	13,72	91,2	0,38
		(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane (Sesquisabinene)	13,81	90,5	0,22
		1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene (α-Curcumene)	14,04	99,3	10,15
		(1S,5S)-2-methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hex-2-ene (7-epi-Sesquithujene)	14,15	96,2	25,41
		Beta-bisabolene	14,26	98,5	5,87
		1,2,3,5,6,7,8,8A-Octahydro- 1, 8A-dimethyl-7-(1-methylethenyl), naphthalene (Eremophilene)	14,36	94,7	2,08
		3-(1,5-Dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-cyclohexene (β-Sesquiphellandrene)	14,42	98,8	12,28
		Nonadecane	14,87	94,6	0,29
		(1R,4R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cuclohex-2-enol (Zingiberenol)	15,15	92,4	0,66
		4-(4-Hydroxy-3-methoxyphenyl)-2-butanone (Zingerone)	15,41	94,1	3,38
		Squalene	15,56	94,8	1,02
		Ethyl ester, hexadecanoic acid	18,04	98,6	3,69
		9(E), 11(E)-Conjugated linoleic acid, ethyl ester	19,97	96,3	4,34
		Ethyl ester, 9-octadecenoic acid	20,04	95,7	2,73
		Dioctyl ester, hexanedioic acid	23,86	97,9	4,79

TR = retention time (minutes)*
P (%) = probability of fitting with mass spectra from NBS and/or NIST libraries*

Table 1. *Total polyphenol levels and GC–MS–identified constituents of ethanol extracts from fresh turmeric and ginger rhizomes.*

Effects of Turmeric and Ginger Extracts on Cell Growth

The cytotoxic activity of ethanol extracts of turmeric and ginger rhizomes was first evaluated in the **MDS-L** myelodysplastic cell line. Compared to ethanol control, as shown in Figure 1A, both extracts reduced cell growth in a dose-dependent manner. Ginger treatment led to a progressive and significant decrease in cell viability, with a marked reduction beginning at 0.012 µg GAE/mL ($p < 0.01$) and reaching nearly complete inhibition at concentrations ≥3.125 µg GAE/mL (Figure 1B, $p < 0.0001$). Turmeric also demonstrated inhibitory effects, although to a lesser extent. A significant reduction was observed even at very low concentrations (0.006 µg GAE/mL; $p < 0.01$), with a gradual decrease in cell growth as the concentration increased (Figure 1C). However, the maximal inhibitory effect of turmeric was slightly lower compared to ginger at equivalent concentrations. Dose–response analysis revealed an IC_{50} of **0.585 µg GAE/mL for ginger and 0.994 µg GAE/mL for turmeric** (Figures 1D and 1E). These data indicate that ginger is more potent than turmeric in suppressing the proliferation of MDS-L cells

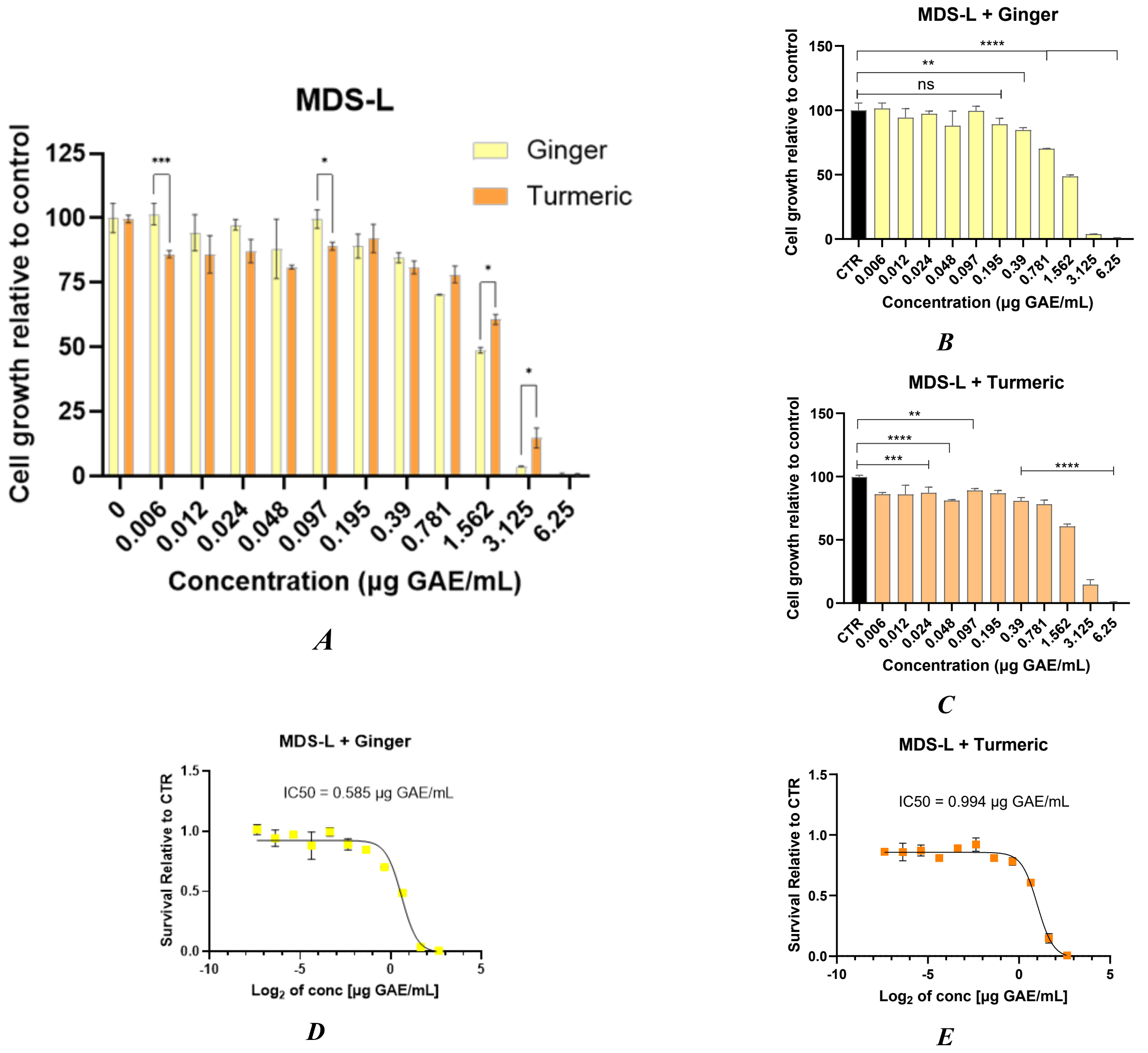


Figure 1: Effect of ginger and turmeric extracts on MDS-L cells. (A) Comparative effect of ginger (yellow bars) and turmeric (orange bars) extracts on MDS-L cells, across a concentration range of 0.0006 – 6.25 µg GAE/mL. (B) Dose-dependent inhibitory effect of ginger showing significant reduction in cell growth starting at 0.012 µg GAE/mL ($p < 0.01$) and reaching nearly complete inhibition at the highest concentrations ($p < 0.0001$). (C) Dose-dependent inhibitory effect of turmeric, with significant decreases observed from 0.006 µg GAE/mL ($p < 0.01$) and a marked reduction at higher concentrations ($p < 0.0001$). (D, E) Dose–response curves and IC_{50} values for ginger (0.585 µg

GAE/mL) and turmeric (0.994 µg GAE/mL), indicating stronger antiproliferative activity of ginger compared to turmeric. Data represent mean ± standard deviation (SD) from three experiments. Statistical significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****), ns = not significant.

To investigate the effects of ginger and turmeric extracts on **MV4-11 AML cells**, these were treated with increasing concentrations of each extract (0.0006 – 6.25 µg GAE/mL). As shown in Figure 2A, both extracts induced a dose-dependent decrease in MV4-11 cell growth. Ginger exhibited a stronger inhibitory effect compared to turmeric, particularly at intermediate concentrations (0.097 – 0.39 µg GAE/mL), where significant differences between the two treatments were observed ($p < 0.05$). When analyzed separately, ginger treatment resulted in a progressive and statistically significant reduction in cell growth starting at 0.024 µg GAE/mL and reaching nearly complete inhibition at the highest concentrations (Figure 2B, $p < 0.0001$). In contrast, turmeric demonstrated a milder effect, with significant reductions only observed at concentrations ≥0.39 µg GAE/mL (Figure 2C, $p < 0.001$). The dose–response curves further confirmed these findings. Ginger displayed a lower IC_{50} value (**0.329 µg GAE/mL**) compared to turmeric (**0.563 µg GAE/mL**), indicating a higher antiproliferative potency of ginger extract on MV4-11 cells (Figures 2D and 2E).

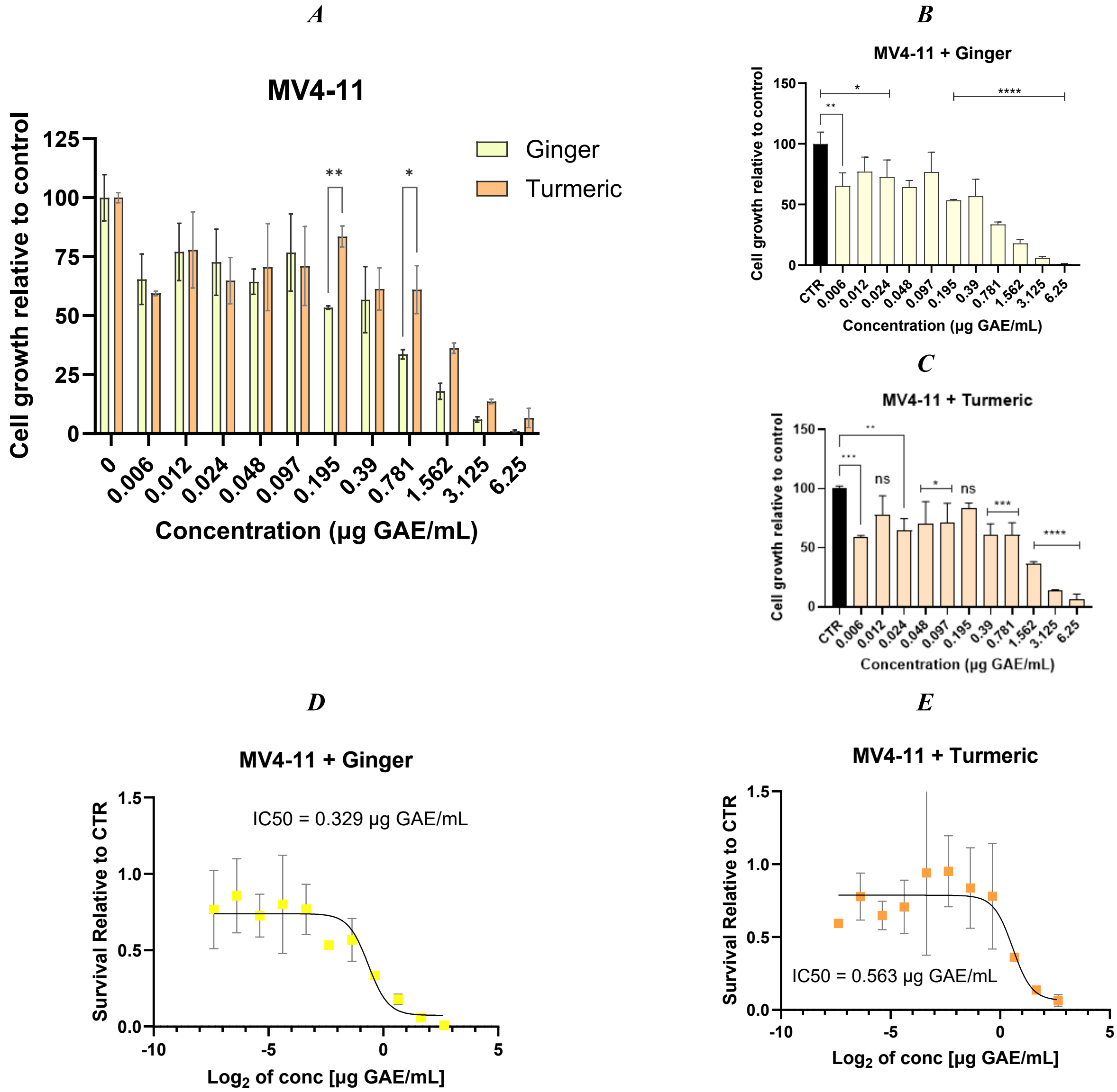
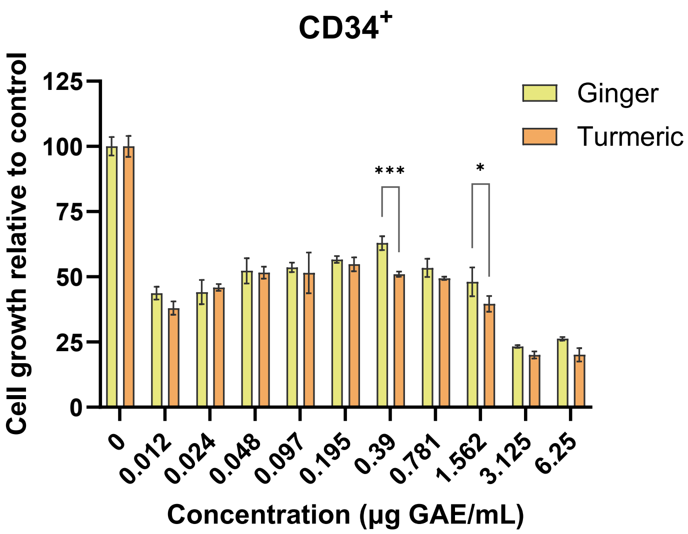


Table 4: *P Figure 2: Effect of ginger and turmeric extracts on the viability of MV4-11 AML cells. (A) MV4-11 AML cells were treated with increasing concentrations (0.006–6.25 µg GAE/mL) of ginger (yellow bars) or turmeric (orange bars)*

in vitro and cell viability was measured using the CellTiter-Glo assay. (B) Dose-dependent inhibitory effect of ginger, showing significant reductions starting at 0.024 µg GAE/mL ($p < 0.05$). (C) Dose-dependent inhibitory effect of turmeric, with significant decreases observed at concentrations ≥0.39 µg GAE/mL ($p < 0.001$). (D, E) Dose–response curves and IC_{50} values for ginger (0.329 µg GAE/mL) and turmeric (0.563 µg GAE/mL), indicating stronger antiproliferative activity of ginger compared to turmeric. Data are presented as mean ± standard deviation (SD) from three experiments. Statistical significance levels: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****), ns = not significant.

To assess potential therapeutic windows for the use of turmeric and/or ginger tracts in AML or MDS, normal **$CD34^+$ hematopoietic stem and progenitor cells** were also exposed to the extracts at different concentration (0.012 – 6.25 µg GAE/mL). Both extracts produced a dose-dependent decrease in $CD34^+$ cell growth (Figure 3A). When analyzed separately, ginger treatment resulted in a progressive and statistically significant reduction in cell viability relative to control, beginning at low concentrations and reaching maximal inhibition at the highest doses (Figure 3B, ****$p < 0.0001$). Turmeric also induced a dose-dependent decrease in cell viability relative to control, with significant effects observed at intermediate and higher concentrations (Figure 3C, ****$p < 0.0001$). Dose–response analysis yielded IC_{50} values of **1.047 µg GAE/mL for ginger and 0.919 µg GAE/mL for turmeric** (Figures 3D–E).

A



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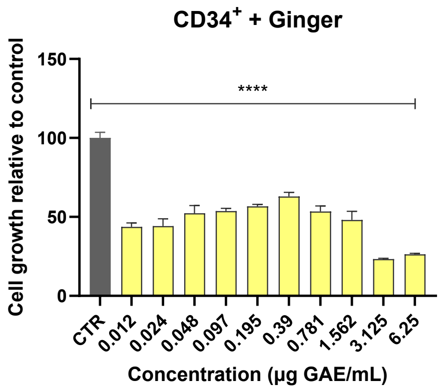

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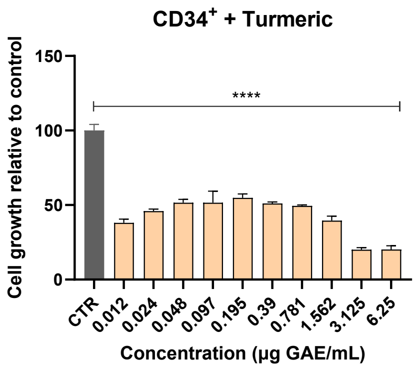


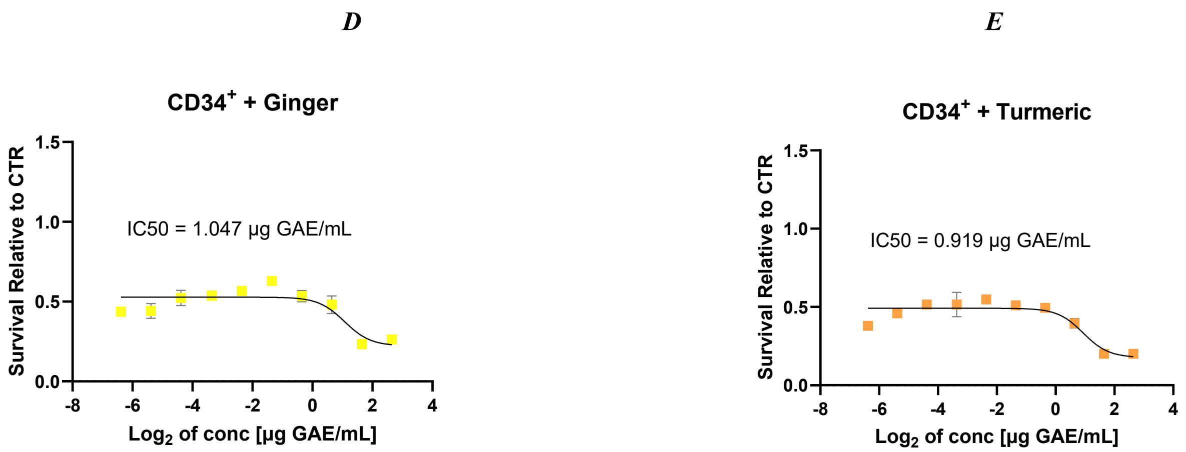
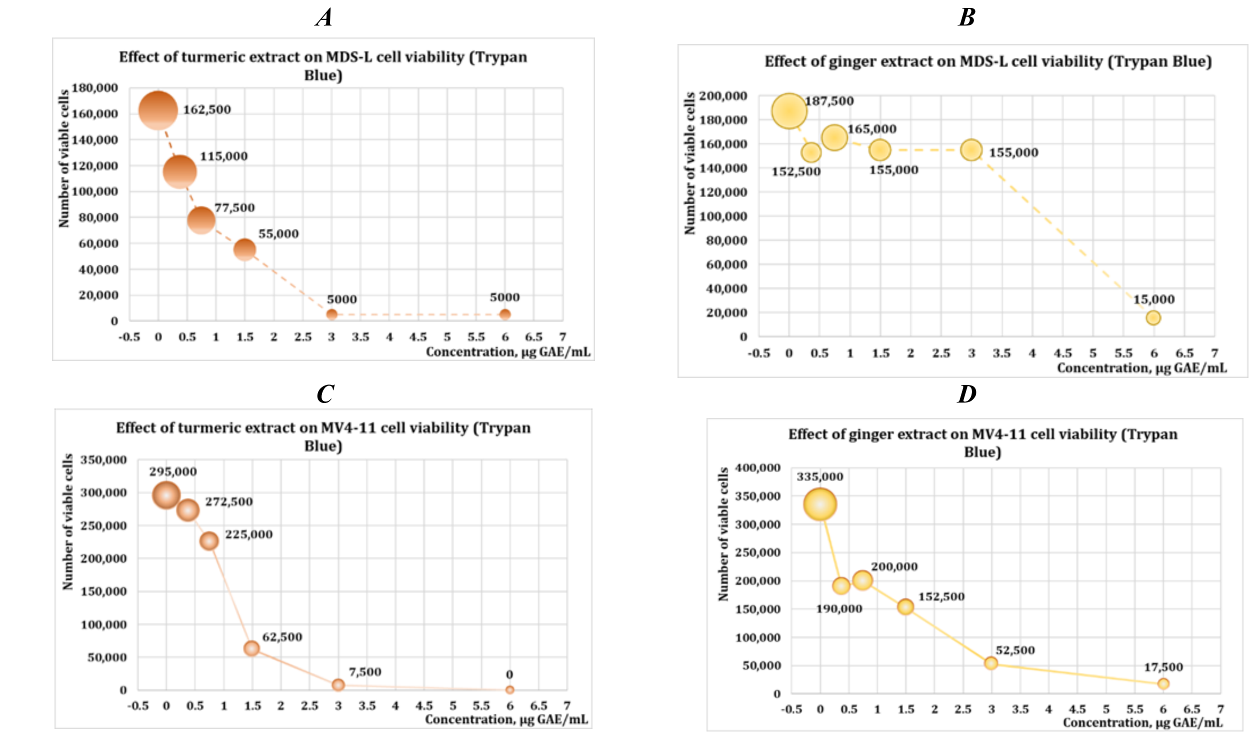


Figure 3: *Effect of ginger and turmeric extracts on the viability of CD34⁺ hematopoietic stem and progenitor cells. (A) CD34⁺ cells were treated with increasing concentrations (0.012 – 6.25 µg GAE/mL) of ginger (yellow bars) or turmeric (orange bars). (B, C) Dose-dependent inhibitory effects of ginger (B) and turmeric (C) on CD34⁺ cell growth relative to the control group (CTR). Statistical comparisons were performed versus CTR. (D, E) Dose–response curves and IC_{50} determination for ginger (D) and turmeric (E). IC_{50} values were 1.047 µg GAE/mL for ginger and 0.919 µg GAE/mL for turmeric. Data are mean ± SD from three experiments. Statistical significance: $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); $p < 0.0001$ (****); ns = not significant.*

To confirm the effects observed with the CellTiter-Glo assay, cell viability was also assessed by the Trypan Blue exclusion method (Figure 4A–F). In both **MDS-L** (Figures 4A–B) and **MV4-11** cells (Figures 4C–D), treatment with turmeric and ginger extracts led to a marked, dose-dependent reduction in viable cell numbers. At concentrations ≥1.5 µg GAE/mL, viability was significantly reduced, and at 6 µg GAE/mL, few or no viable cells remained. A similar pattern was observed in **CD34⁺ hematopoietic progenitor cells** (Figures 4E–F), where higher concentrations of both extracts (3 and 6 µg GAE/mL) produced a substantial decline in viability. These results indicate that while the extracts are effective against malignant cells, their cytotoxicity extends to normal hematopoietic cells at elevated concentrations.



E *F*

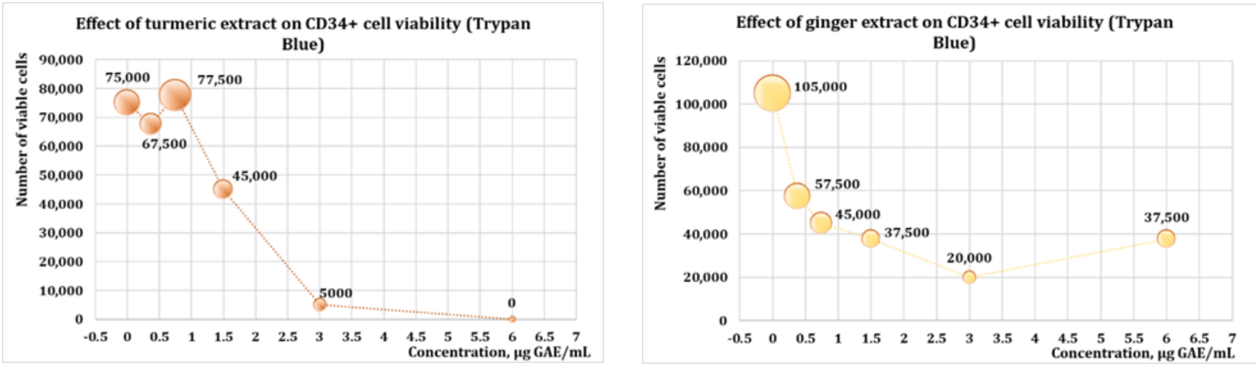


Figure 4: *The effect of turmeric and ginger ethanol extracts on the viability of MDS-L (A–B), MV4-11 (C–D) and CD34⁺ (E–F) cells, assessed by Trypan Blue exclusion assay. Cells were seeded in 24-well plates and treated separately with increasing concentrations of turmeric (A, C, E) or ginger (B, D, F) extracts (0, 0.375, 0.75, 1.5, 3 and 6 µg GAE/mL) for 72 h. Cell viability was determined by Trypan Blue exclusion. Each circle represents the mean number of viable cells at the indicated concentration, and the circle size is proportional to the cell count.*

To evaluate whether the presence of the bone marrow microenvironment modulates the cytotoxic activity of the extracts, viability was tested in **co-cultures of primary human MSCs with MDS-L or MV4-11 cells** using the Trypan Blue exclusion assay. Treatment with turmeric extract resulted in a clear, dose-dependent reduction in viable cell numbers (Figure 5A–B). Significant cytotoxicity was observed at concentrations ≥1.5 µg GAE/mL. Compared with monocultures, co-cultures initially showed partial protection from extract-induced cell death, consistent with a supportive effect of the stromal layer. However, this protective barrier was overcome at higher concentrations, where viability was markedly reduced in both leukemic models.

A *B*

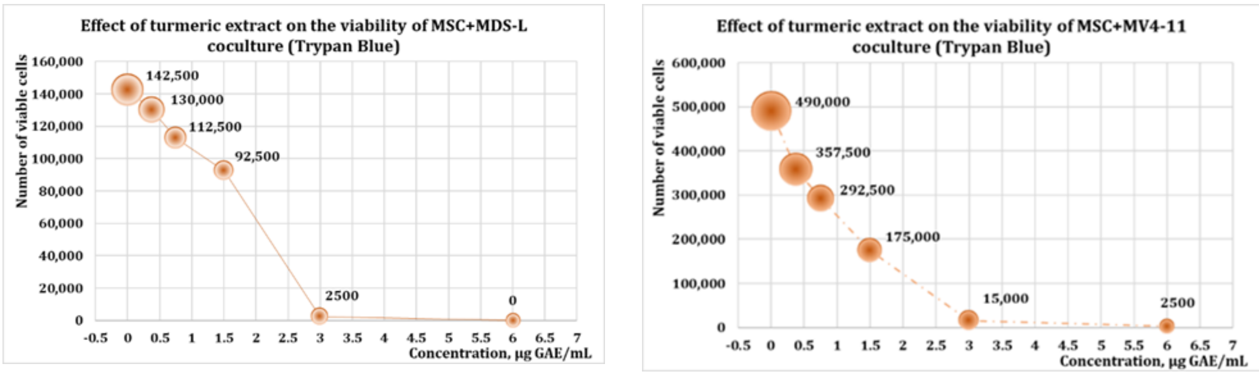


Figure 5: *Effect of ethanolic turmeric extract on the viability of MSC + MDS-L (A) and MSC + MV4-11 (B) cocultures assessed by Trypan Blue exclusion assay. Cocultures of MSC with MDS-L (A) or MV4-11 (B) cells were established in 24-well plates and treated with increasing concentrations of turmeric extract (0, 0.375, 0.75, 1.5, 3 and 6 µg GAE/mL) for 72 h. Cell viability was determined by Trypan Blue exclusion. Each circle represents the mean number of viable cells at the indicated concentration, and the size of the circle is proportional to the number of viable cells.*

Effects of Turmeric and Ginger Extracts on Apoptosis

To determine whether the decreased cell growth seen during treatment with turmeric and ginger extracts were mediated through cell death and apoptosis, flow cytometry was performed on malignant and normal hematopoietic cells. In MSC + MDS-L cocultures, treatment with turmeric extract induced a progressive, concentration-dependent increase in apoptosis (Figure 6A–F). While most cells in the control group remained viable, low concentrations (0.375–0.75 µg GAE/mL) produced a modest rise in late apoptotic events. At higher concentrations (1.5–6 µg GAE/mL), the majority of cells shifted into late apoptosis, confirming a strong pro-apoptotic effect of turmeric on MDS-L cells, even in the presence of MSCs.

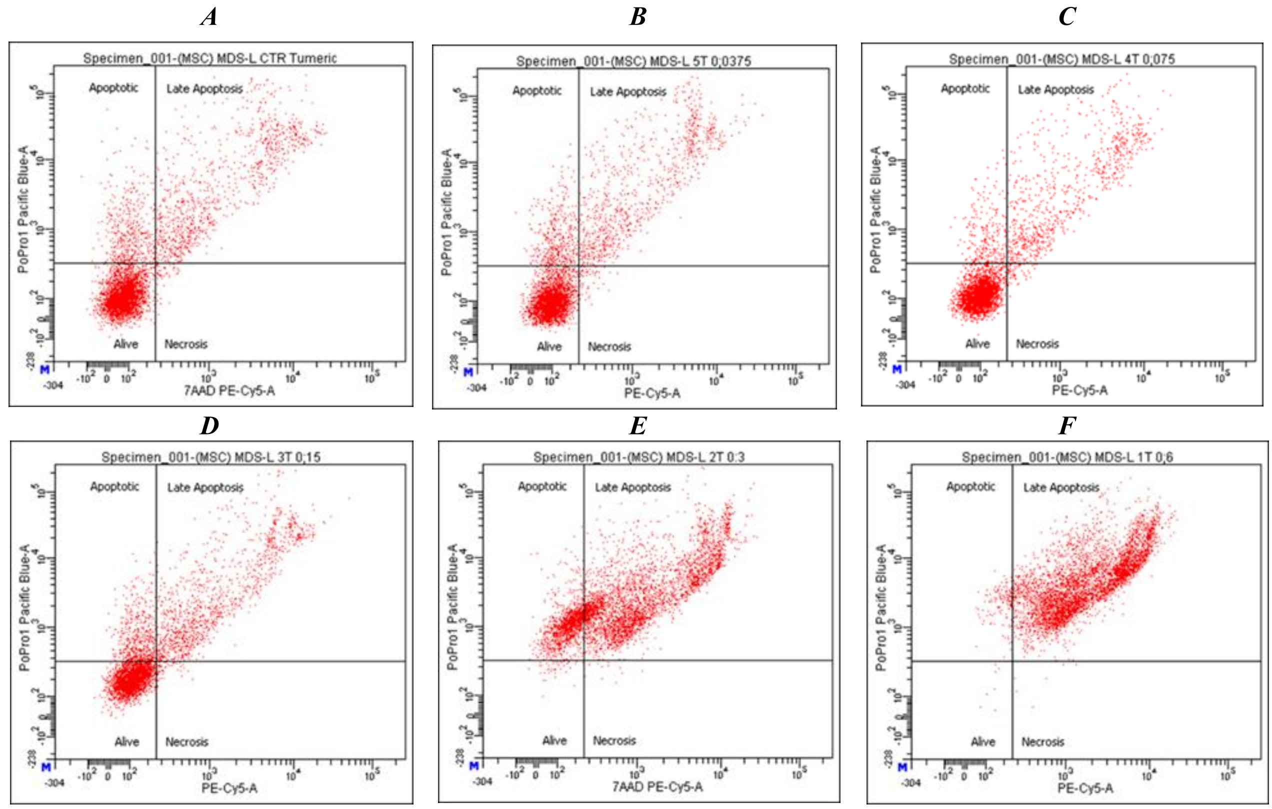
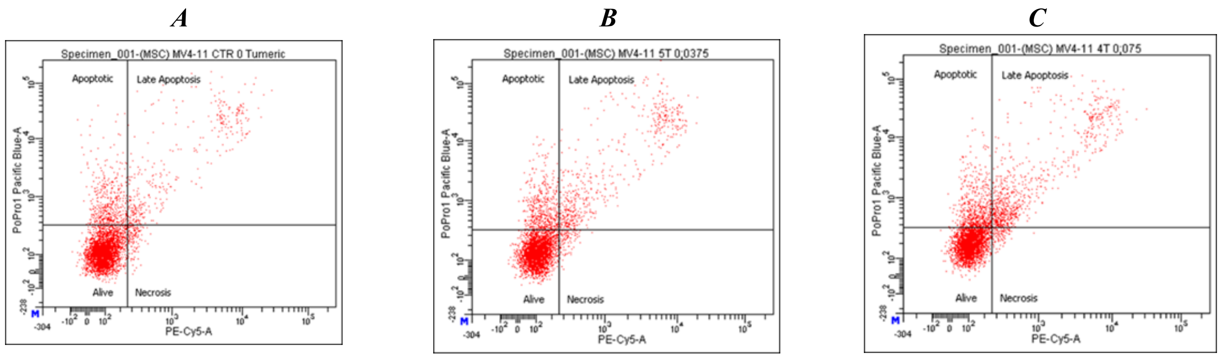


Figure 6: *Flow cytometric analysis of apoptosis in MSC + MDS-L coculture following treatment with different concentrations of ethanolic turmeric extract considered as gallic acid equivalents (GAE): control (a), 0.375 µg GAE/ mL (b), 0.75 µg GAE/ mL (c), 1.5 µg GAE/ mL (d), 3 µg GAE/ mL (e), 6 µg GAE/ mL (f). Data shows one representative experiment from three with similar results*

Similarly, in MSC + MV4-11 cocultures, turmeric extract triggered a clear dose-dependent apoptotic response (Figure 7A–F). Control samples contained mostly viable cells, whereas treatment with intermediate concentrations (1.5–3 µg GAE/mL) significantly increased late apoptosis. At the highest concentration tested (6 µg GAE/mL), most cells were apoptotic, confirming robust induction of programmed cell death in AML cells within the stromal microenvironment.



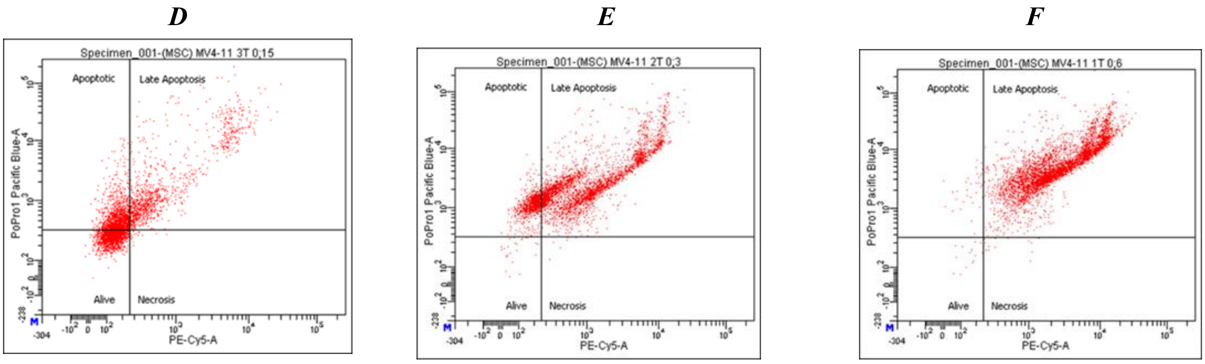


Figure 7: *Flow cytometric analysis of apoptosis in MSC + MV4-11 coculture following treatment with different concentrations of ethanolic turmeric extract considered as gallic acid equivalents (GAE): control (a), 0.375 µg GAE/ mL (b), 0.75 µg GAE/ mL (c), 1.5 µg GAE/ mL (d), 3 µg GAE/ mL (e), 6 µg GAE/ mL (f). Data shows one representative experiment from three with similar results.*

For normal CD34+ hematopoietic progenitor cells, turmeric extract also induced apoptosis in a dose-dependent manner (Figure 8A–F). Low concentrations (0.375–0.75 µg GAE/mL) had minimal impact, whereas intermediate concentrations (1.5–3 µg GAE/mL) caused a gradual increase in apoptotic cells, predominantly in the late stage. At 6 µg GAE/mL, apoptosis was markedly increased, demonstrating that while effective against leukemic cells, turmeric also affects normal progenitors at higher doses.

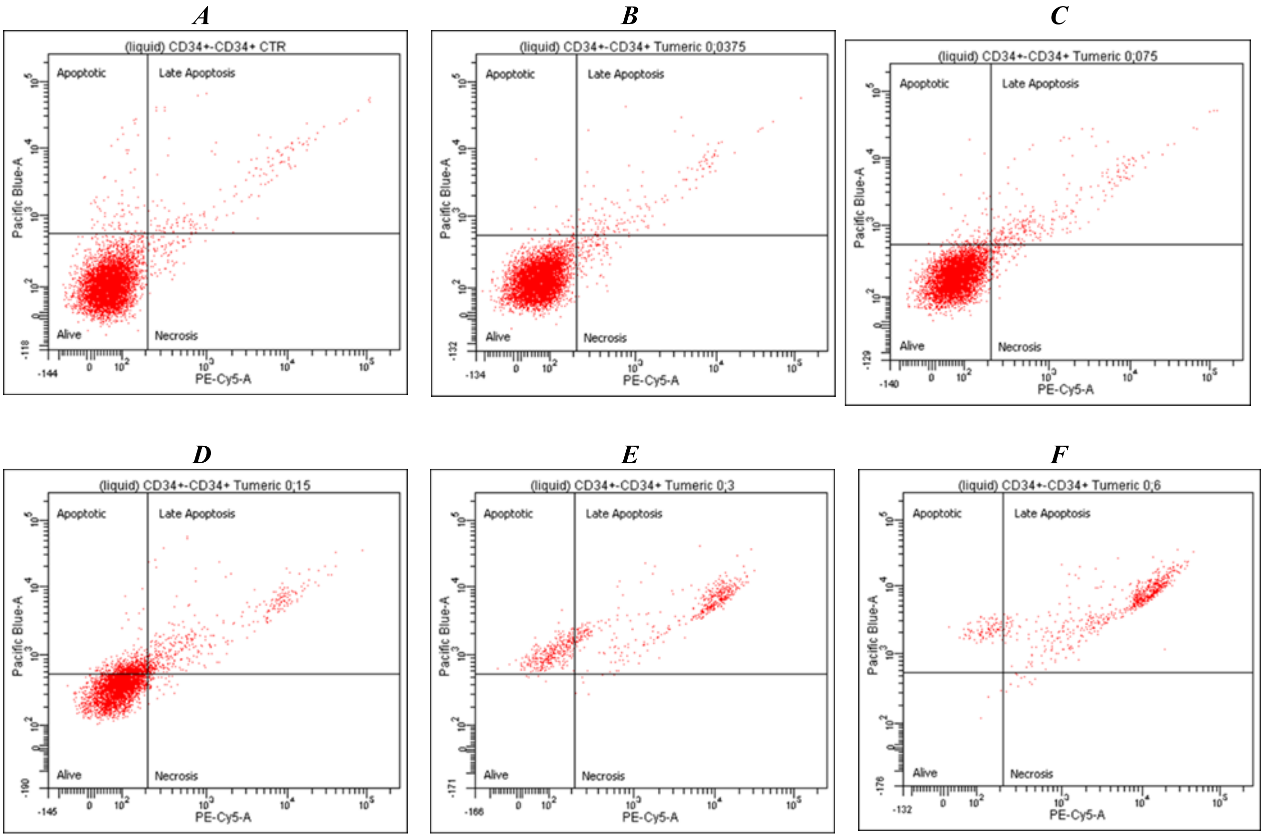


Figure 8: *Flow cytometric analysis of apoptosis in CD34+ cells following treatment with different concentrations of ethanolic turmeric extract considered as gallic acid equivalents (GAE): control (a), 0.375 µg GAE/ mL (b), 0.75 µg*

GAE/ mL (c), 1.5 µg GAE/ mL (d), 3 µg GAE/ mL (e), 6 µg GAE/ mL (f). Data shows one represetnative experiment from three with similar results.

Treatment with ginger extract produced a similar but less pronounced apoptotic effect in CD34⁺ cells (Figure 9A–F). Control and low concentrations (0.375–0.75 µg GAE/mL) showed little change compared with baseline. Apoptosis increased modestly at intermediate concentrations (1.5–3 µg GAE/mL), but plateaued at 6 µg GAE/mL, suggesting no further enhancement at higher doses. Compared to turmeric, ginger extract appeared slightly less toxic to CD34⁺ cells, while maintaining cytotoxicity against malignant counterparts.

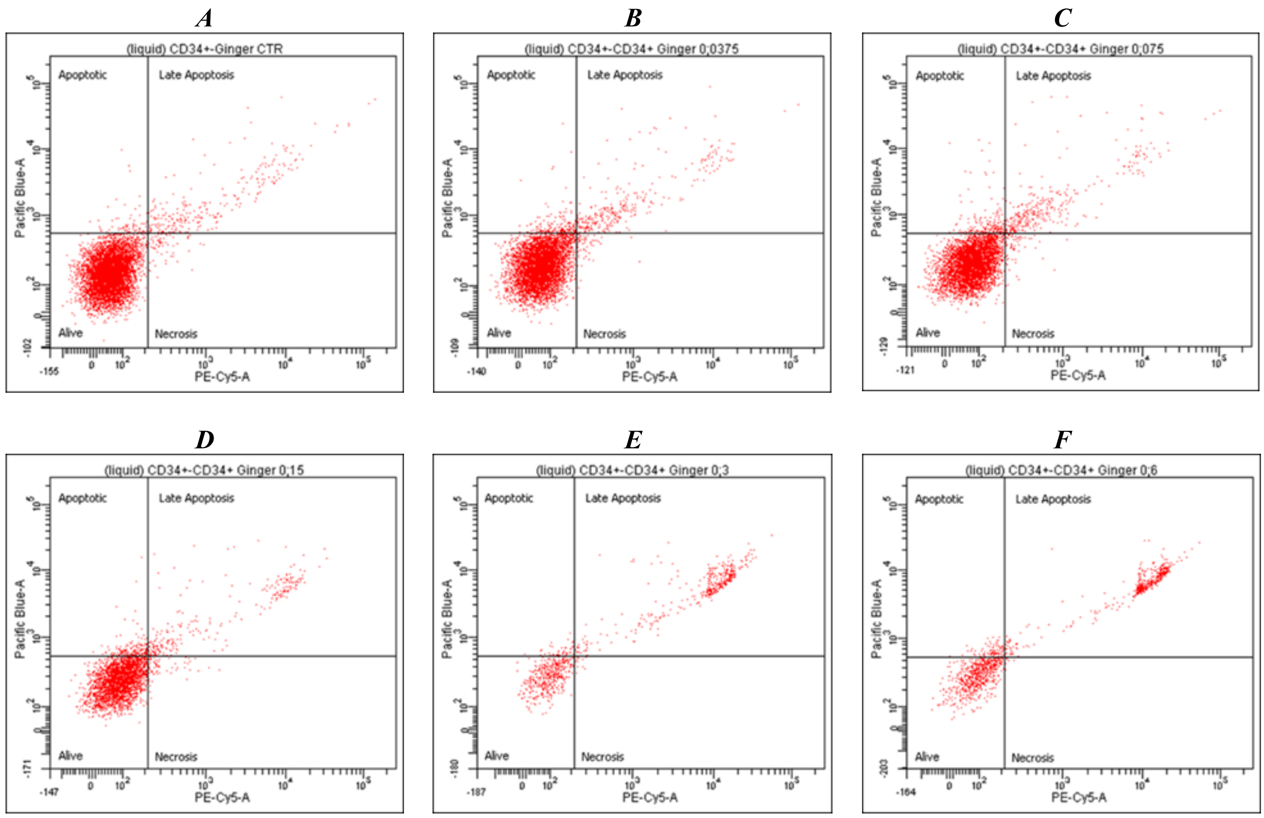


Figure 9: *Flow cytometric analysis of apoptosis in CD34+ cells following treatment with different concentrations of ethanolic ginger extract considered as gallic acid equivalents (GAE): control (a), 0.375 µg GAE/ mL (b), 0.75 µg GAE/ mL (c), 1.5 µg GAE/ mL (d), 3 µg GAE/ mL (e), 6 µg GAE/ mL (f). Data shows one representative experiment from three with similar results.*

Taken together, these results confirm that both turmeric and ginger extracts exert their anti-leukemic activity primarily by inducing apoptosis in malignant hematopoietic cells. Importantly, the data also suggest the existence of a therapeutic window where these extracts preferentially target MDS-L and MV4-11 cells while sparing normal CD34⁺ progenitors at lower concentrations.

Discussions

The results obtained in this study demonstrate differences between turmeric and ginger extracts, both in their phytochemical composition and in their biological activity on malignant and normal hematopoietic cells. From a phytochemical perspective, the ethanolic extract of *Curcuma longa* rhizomes displayed a higher total polyphenol content (1980 ± 37.62 mg GAE/L) compared with *Zingiber officinale* (91 ± 1.82 mg GAE/L). This disparity aligns with previous reports. For instance, Danciu et al. (15) reported 182 mg GAE/g for turmeric versus 16 mg GAE/g for ginger under comparable conditions, highlighting the statistically significant difference between the two species ($p < 0.01$). Bouchama et al. (16) confirmed higher levels of polyphenols and flavonoids in turmeric across several solvents (water, ethanol, methanol). Together, these findings underscore the phytochemical richness of turmeric.

GC–MS analysis revealed distinct chemical fingerprints for the two extracts. In turmeric, tumerone (35.43%) and curlone (19.90%) were dominant, both recognized for antiproliferative and pro-apoptotic activity (17). In ginger, 7-epi-sesquithujene (25.41%) and β-sesquiphellandrene (12.28%) were prevalent, pointing to potentially different mechanisms of action compared to the better studied curcumin and gingerol. Surprisingly, despite its lower polyphenol content, ginger extract demonstrated greater cytotoxicity, reflected by lower IC_{50} values in both MDS-L and MV4-11 cells. This suggests that antitumor activity is not dictated solely by phenolic content, but rather by specific chemical profiles. Sesquiterpenes such as 7-epi-sesquithujene and β-sesquiphellandrene, though less well studied, may contribute substantially. Zehra et al. (18) reported that sesquiterpenes can trigger apoptosis and inhibit migration through mitochondrial regulation and suppression of invasion-related proteins, including MMP-2 and MMP-9, supporting this interpretation.

Conversely, the major compounds of turmeric, tumerone and curlone, have documented antiproliferative properties (17), but their activity may be influenced by stability, bioavailability, or interactions with other extract constituents. This could partially explain why turmeric, despite a richer polyphenolic composition, did not always outperform ginger in our assays.

Functional studies confirmed the cytotoxic effects of both extracts. The CellTiter-Glo and Trypan Blue assays demonstrated dose-dependent reductions in cell growth and cell viability, consistent with damage to cell membrane integrity. These results are supported by Lee et al. (19), who reported increased cell death in oxaliplatin-resistant HCT-116 cells after treatment with fermented ginger extract, validating Trypan Blue as a complementary readout.

The bone marrow stromal microenvironment was also considered. Co-culture experiments revealed that MSCs conferred some protection at low extract concentrations, in line with previous studies showing that stromal cells enhance leukemic survival via paracrine and contact-dependent signals (20). Importantly, this protective effect was abrogated at higher doses of the extracts, which effectively suppressed leukemic viability. This suggests that turmeric and ginger can not only target malignant cells directly, but may also counteract stromal-mediated resistance — an important consideration in diseases such as MDS and AML, where the microenvironment significantly influences therapeutic response (21). Thus, the ability of extracts to counteract this cellular protection suggests a new strategy in studying the effect of natural compounds, not only on tumor cells, but also on their interactions with their stromal microenvironment.

Flow cytometry provided additional insight into cell death mechanisms. Turmeric extract induced a progressive increase in apoptosis in both MDS-L and MV4-11 cells, including in MSC co-culture, confirming its pro-apoptotic potential. This aligns with prior findings that turmeric-derived compounds can modulate NF-κB and PI3K/Akt/mTOR pathways, driving apoptosis and suppressing inflammation (22). In normal $CD34^+$ progenitors, both extracts induced apoptosis at higher concentrations, but effects were less pronounced than in leukemic cells, suggesting a therapeutic window. Reports from Yue et al. (17) and Bistué-Rovira et al. (23) similarly describe a selective profile, with Curcuma-derived fractions reducing malignant proliferation while sparing normal hematopoietic cells.

Altogether, these findings emphasize that turmeric and ginger extracts exert dose- and context-dependent effects on malignant hematopoietic cells, including in the stromal niche. Both extracts induced apoptosis and suppressed viability, while having more moderate effects on normal $CD34^+$ cells. The observation that ginger exhibited greater potency despite lower polyphenolic content points toward underexplored bioactive sesquiterpenes as promising candidates for further study.

Conclusions and Perspectives

This study highlights the antitumor potential of *Curcuma longa* and *Zingiber officinale* extracts in pre-leukemic and leukemic contexts. Despite higher phenolic content in turmeric, ginger displayed stronger cytotoxic effects under certain conditions, suggesting distinct underlying mechanisms. Both extracts counteracted the protective influence of MSCs, underscoring their ability to target not only malignant cells but also their microenvironment. Importantly, their more moderate effects on normal $CD34^+$ cells suggest a degree of therapeutic selectivity. These data justify continued exploration of turmeric and ginger extracts as complementary agents in hematological oncology, with future work needed to define molecular mechanisms, evaluate efficacy in animal models, and ultimately test standardized formulations in clinical settings.

Ethics Statement and Conflict of Interest Disclosures

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Conflict of interest: No known conflict of interest correlated with this publication.

Availability of data and materials: The data used and/ or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interests: The authors declared that they have no competing interests.

The use of generative AI and AI-assisted technologies: The authors did not use in this article generative AI and AI-assisted technologies.

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