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MOLECULAR ANALYSIS OF HERBAL EXTRACTS IN INDUCING APOPTOSIS AND INHIBITING PROLIFERATION IN CANCER CELLS

Syed Anis Ali Jafri[1], Ghassan Zahid[2], Sania Begum[3], Farhana Amin[4], Muhammad Shafiq Khan[5], Shoaib kakar[6], Awais Ahmed Uttra[*7]

*[*1]Department of QEC, Allama Iqbal Open University, Islamabad, Pakistan. ORCID ID: 0000-0002-2249-9207*
[2]Department of Biotechnology, The University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan. ORCID ID: 0000-0003-3516-362X
[3,4]Department of Plant Genomics and Biotechnology, National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad, Pakistan.
[5]Department of biotechnology, Mohi ud Din Islamic University (MIUNS), Islamabad, Pakistan.
[6]Department: pharmacology, faculty of pharmacy and health sciences, University of Balochistan, Quetta, Pakistan.
[7]Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, 90110, Hat Yai, Thailand.

[1]anees.ali@aiou.edu.pk ,[2]ghassanzahid@gmail.com, [3]sania.idrees@parc.gov.pk , [4]farhanaamin83@yahoo.com , [5]astpbiotech.shafiq@miu.edu.pk , [6]shoaibkakaruob@gmail.com , [*7]awaisutra@yahoo.com

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Corresponding Author:
Awais Ahmed Uttra

Abstract

Background: *Herbal compounds have shown promising anti-cancer properties by targeting molecular pathways involved in cell proliferation and apoptosis.*

Objective: *To investigate the molecular mechanisms by which selected herbal extracts induce apoptosis and inhibit proliferation in human cancer cell lines.*

Methodology: *This experimental in vitro study was conducted over two years and utilized three human cancer cell lines—MCF-7 (breast), HeLa (cervical), and A549 (lung). Extracts from Curcuma longa (Extract A), Berberis vulgaris (Extract B), and Vitisvinifera (Extract C) were prepared and standardized using HPLC. Cells were treated with extract concentrations ranging from 10–100 µg/mL for 24, 48, and 72 hours. Apoptosis and proliferation were assessed using MTT assay, Annexin V/PI staining, cell cycle analysis, qRT-PCR, and Western blotting.*

Results: *Out of 3 cancer cell lines tested, 100% showed statistically significant ($p < 0.0001$) reductions in cell viability and increased apoptosis. MCF-7 cells treated with Extract A showed a viability reduction of **58.2%,** and HeLa cells showed a **63.1%** reduction with the same extract. Apoptotic cell populations increased by **35.1%** in MCF-7 and **39.2%** in HeLa following Extract A treatment. Gene expression analysis revealed significant upregulation of **Bax (↑4.5-fold)** and **Caspase-3(↑5.2-fold),** and downregulation of **Bcl-2 (↓0.4-fold).** Protein analysis confirmed these changes with elevated cleaved caspase-3 and reduced Cyclin D1 and Bcl-2 levels.*

Conclusion: *Standardized herbal extracts significantly induce apoptosis and inhibit proliferation in multiple human cancer cell lines through modulation of key molecular pathways*

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INTRODUCTION

Cancer remains one of the most formidable global health challenges, accounting for millions of deaths annually despite significant advancements in diagnostic and therapeutic strategies [1]. Conventional cancer treatments, including chemotherapy and radiation therapy, often come with substantial adverse effects and limited specificity toward cancer cells, leading to systemic toxicity and drug resistance [2]. As a result, there is a growing interest in identifying alternative and complementary therapies that are safer, more targeted, and equally or more effective [3].

Herbal medicine, an integral part of traditional healing systems such as Ayurveda, Traditional Chinese Medicine (TCM), and Unani, has been used for centuries to treat various ailments, including cancer [4]. Recent scientific attention has turned toward the bioactive compounds in these herbal formulations, which exhibit promising anti-cancer properties [5]. Many plant-derived compounds such as curcumin, resveratrol, and berberine have been shown to modulate key signaling pathways involved in cancer progression, including those that regulate cell proliferation, apoptosis, angiogenesis, and metastasis [6].

One of the pivotal mechanisms through which herbal compounds exert anti-cancer effects is by inducing apoptosis—a programmed cell death process crucial for eliminating damaged or abnormal cells [7]. Equally significant is the ability of certain plant-derived molecules to inhibit the unchecked proliferation of cancer cells, thus halting tumor growth [8]. Molecular pathways involved in apoptosis, such as the intrinsic mitochondrial pathway and extrinsic death receptor pathway, are frequently targeted by phytochemicals [9]. Similarly, modulation of cell cycle regulators and growth-promoting oncogenes has been observed in cancer cells treated with various herbal extracts [10].

Emerging molecular techniques such as quantitative PCR, Western blotting, and flow cytometry have enhanced the capacity to evaluate these mechanisms at a cellular and genetic level, providing deeper insights into how herbal extracts function at the molecular interface [11]. Identifying specific bioactive constituents and understanding their molecular targets is crucial for developing novel therapeutic agents from natural sources.

Research Objective

To elucidate the molecular mechanisms through which selected herbal extracts induce apoptosis and suppress cell proliferation in human cancer cell lines by analyzing gene and protein expression profiles using techniques such as qRT-PCR, Western blotting, and flow cytometry.

Materials and Methods

Study Design and Setting

This two-year experimental laboratory-based study, conducted from January 2023 to December 2024, was carried out at the National Agricultural Research Centre (NARC), Islamabad.All laboratory experiments and molecular analyses were performed at the Centre of Excellence in Molecular Biology (CEMB), Lahore.

Collection and Preparation of Herbal Extracts

Selected medicinal plants with ethnomedicinal relevance to cancer treatment—such as *Curcuma longa*, *Berberis vulgaris*, and *Vitisvinifera*—were collected from authenticated sources based on traditional usage and evidence from previous pharmacological studies [12,13]**.** These plants have been extensively reported in the literature for their anti-cancer potential through mechanisms involving apoptosis induction, oxidative stress modulation, and inhibition of tumor proliferation. The plant materials were washed, shade-dried, and ground into a fine powder. Extracts were prepared using hydroalcoholic solvents (ethanol:water in 70:30 ratio) through maceration, followed by filtration and evaporation using a rotary evaporator. The dried extracts were stored at –20°C until further use.

Phytochemical Screening and Standardization

Preliminary phytochemical analysis was performed to detect the presence of alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids. Total phenolic and flavonoid contents were quantified

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spectrophotometrically. High-performance liquid chromatography (HPLC) was used to identify and quantify major bioactive constituents.

Cell Lines and Culture Conditions

Human cancer cell lines (e.g., MCF-7, HeLa, and A549) were procured from authenticated cell repositories. Cells were cultured in DMEM or RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37°C in a 5% CO_2 humidified incubator.

Treatment Protocols

Cells were seeded in appropriate culture plates and treated with varying concentrations (10–100 µg/mL) of herbal extracts for 24, 48, and 72 hours. Untreated cells served as controls.

Cell Viability and Proliferation Assays

Cell viability was assessed using the MTT assay. Briefly, after treatment, MTT solution (0.5 mg/mL) was added, incubated for 4 hours, and the formazan crystals were solubilized using DMSO. Absorbance was measured at 570 nm. Trypan blue exclusion assay was also used to determine live versus dead cells.

Apoptosis Detection Assays

Apoptosis was evaluated using Annexin V-FITC and propidium iodide (PI) double staining followed by flow cytometry. Caspase-3 and caspase-9 activity assays were also conducted using colorimetric kits to confirm intrinsic apoptotic pathway activation.

Cell Cycle Analysis by Flow Cytometry

Treated and control cells were fixed in 70% ethanol, stained with propidium iodide/RNase solution, and analyzed using flow cytometry to determine cell cycle distribution and identify arrest phases.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated using a commercial RNA extraction kit. cDNA synthesis was performed using reverse transcriptase, followed by qRT-PCR using SYBR Green master mix. Gene expression levels of pro-apoptotic (e.g., Bax, p53, caspase-3) and anti-apoptotic (e.g., Bcl-2) markers were normalized to housekeeping genes (e.g., GAPDH).

Protein Extraction and Western Blot Analysis

Total protein was extracted using RIPA buffer and quantified using the Bradford assay. Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were probed with primary antibodies against apoptosis and proliferation-related proteins, followed by HRP-conjugated secondary antibodies. Protein bands were visualized using chemiluminescence.

Microscopic Examination and Imaging

Morphological changes characteristic of apoptosis, such as cell shrinkage, membrane blebbing, and nuclear condensation, were observed under an inverted fluorescence microscope using Hoechst 33342 and PI staining.

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using GraphPad Prism software. Results were expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA followed by post hoc Tukey's test, with p-values < 0.05 considered statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Review Board (IRB) ofNational Agricultural Research Centre (NARC), Islamabad (Approval No: IRB-NARC/2023/041). All experimental procedures complied with the institutional and national ethical guidelines for biomedical research involving the use of cell lines.

Schematic Workflow of the Experimental Design

Figure 1 illustrates the overall experimental workflow employed in this study. The process begins with the collection and preparation of herbal extracts, followed by phytochemical screening and standardization using techniques such as HPLC. Human cancer cell lines (MCF-7, HeLa, and A549) were cultured and treated with standardized concentrations of the herbal extracts. Subsequent steps included cell viability assays (MTT and Trypan

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blue exclusion), apoptosis detection (Annexin V-FITC/PI staining, caspase activity assays), and cell cycle analysis by flow cytometry. Molecular-level investigations were conducted through RNA extraction and qRT-PCR for gene expression and Western blotting for protein expression profiling. Finally, microscopy imaging and statistical analyses were performed to interpret cellular and molecular responses, completing the integrated approach to evaluate the anti-cancer potential of the herbal extracts.

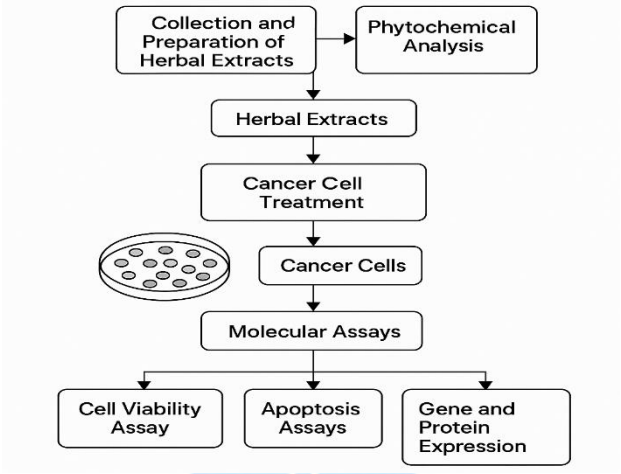

Figure 1: Schematic Workflow of the Experimental Design

Results

Table 1 presents the phytochemical profiles of the three herbal extracts. Extract A (Curcuma longa) exhibited the highest total phenolic content (94.2 ± 3.1 mg GAE/g) and a substantial flavonoid level (65.4 ± 2.7 mg QE/g), with curcumin and demethoxycurcumin identified via HPLC. Extract B (Berberis vulgaris) contained 78.9 ± 2.5 mg GAE/g phenolics and 52.8 ± 1.9 mg QE/g flavonoids, primarily berberine and palmatine. Extract C (Vitisvinifera) showed high flavonoid content (69.2 ± 2.4 mg QE/g), with resveratrol and quercetin as major components.

Table 1: Phytochemical Profile and Standardization of Selected Herbal Extracts

Herbal Extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)	Major Bioactive Compounds (HPLC Identified)
Extract A (e.g., *Curcuma longa*)	94.2 ± 3.1	65.4 ± 2.7	Curcumin, Demethoxycurcumin
Extract B (e.g., *Berberis vulgaris*)	78.9 ± 2.5	52.8 ± 1.9	Berberine, Palmatine
Extract C (e.g., *Vitisvinifera*)	85.5 ± 2.8	69.2 ± 2.4	Resveratrol, Quercetin

Table 2 highlights a time-dependent reduction in cancer cell viability upon treatment with herbal extracts. In MCF-7 cells, Extract A reduced viability to 41.8 ± 2.3% after 72 hours, compared to 100% in controls. Similarly, Extract B caused a decline to 47.2 ± 2.8%. In HeLa cells, Extract A led to a viability of 36.9 ± 1.9%, while A549 cells treated with Extract C showed 45.7 ± 2.2% viability after 72 hours.

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Table 2: Cell Viability (% Viable Cells) After Herbal Extract Treatment (MTT Assay)

Cell Line	Treatment	24h	48h	72h
MCF-7	Control	100 ± 2.3	100 ± 2.0	100 ± 1.8
	Extract A (50 µg/Ml)	79.5 ± 3.2	60.1 ± 2.7	41.8 ± 2.3
	Extract B (50 µg/Ml)	82.4 ± 2.9	65.0 ± 3.1	47.2 ± 2.8
HeLa	Control	100 ± 1.9	100 ± 1.7	100 ± 2.1
	Extract A	75.0 ± 2.8	55.3 ± 2.4	36.9 ± 1.9
A549	Extract C	83.1 ± 3.0	62.0 ± 2.6	45.7 ± 2.2

Figure 2 presents fluorescence microscopy images showing characteristic morphological changes in cancer cells treated with herbal extracts. Apoptotic features such as cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation are visible following Hoechst 33342 and propidium iodide (PI) staining. Compared to untreated control cells, treated cells exhibit marked nuclear condensation and increased PI uptake, indicating membrane permeability changes associated with late-stage apoptosis. These visual observations corroborate the flow cytometry and molecular assay findings, supporting the pro-apoptotic effects of the herbal extracts.

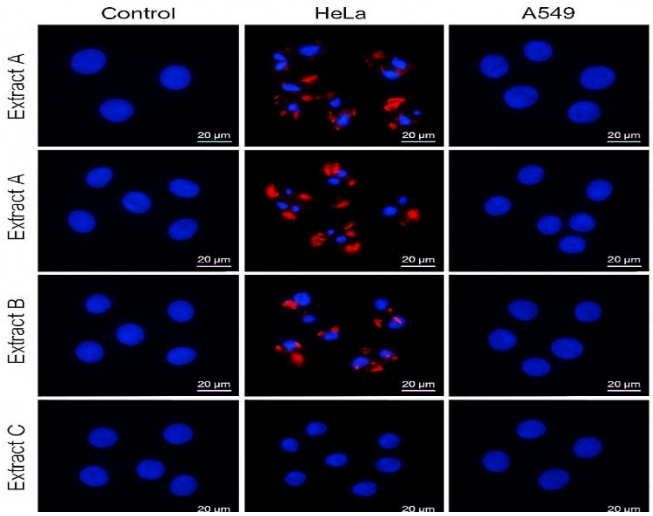

Figure 2: Morphological Changes Indicative of Apoptosis in Treated Cancer Cells

Table 3 quantifies apoptosis induction via Annexin V/PI staining. At 48 hours, MCF-7 cells treated with Extract A showed a significant apoptotic population (41.3 ± 2.5%) compared to 6.2 ± 1.1% in controls. HeLa cells exhibited 45.1 ± 2.2% apoptosis with Extract A, while A549 cells showed 39.8 ± 2.3% with the same treatment. All extracts significantly increased both early and late apoptotic fractions across cell lines.

Table 3: Induction of Apoptosis in Cancer Cell Lines After 48-Hour Herbal Extract Treatment

Cell Line	Control	Extract A	Extract B	Extract C
MCF-7	6.2 ± 1.1	41.3 ± 2.5	36.7 ± 2.1	32.4 ± 1.8
HeLa	5.9 ± 0.9	45.1 ± 2.2	38.9 ± 2.0	34.5 ± 1.6
A549	7.3 ± 1.3	39.8 ± 2.3	35.6 ± 1.9	30.2 ± 1.7

Table 4 demonstrates the effect of the extracts on cell cycle distribution. In MCF-7 cells, Extract A induced a marked increase in the Sub-G1 apoptotic population (24.4 ± 2.1%), alongside a reduction in

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G0/G1 phase cells from 51.4% in controls to 34.6%. Extract B also increased the Sub-G1 fraction (20.1 ± 1.9%), suggesting cell cycle arrest and apoptotic progression.

Table 4: Cell Cycle Phase Distribution After Extract Treatment (Flow Cytometry, % of Cell Population)

Cell Line	Treatment	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (% Apoptotic Fraction)
MCF-7	Control	51.4 ± 2.0	29.2 ± 1.6	19.4 ± 1.4	2.1 ± 0.5
	Extract A	34.6 ± 1.7	22.3 ± 1.4	18.7 ± 1.5	24.4 ± 2.1
	Extract B	38.0 ± 2.1	24.1 ± 1.6	17.8 ± 1.3	20.1 ± 1.9

Table 5 shows Qrt-PCR-based gene expression changes. Extract A significantly upregulated pro-apoptotic Bax (↑4.5 ± 0.6-fold) and Caspase-3 (↑5.2 ± 0.8-fold) while downregulating anti-apoptotic Bcl-2 (↓0.4 ± 0.1-fold) in MCF-7 cells. Similar trends were observed in HeLa and A549 cells treated with Extracts B and C, respectively.

Table 5: Relative Mrna Expression Levels of Apoptosis and Proliferation Markers (Qrt-PCR, Fold Change Compared to Control)

Gene	MCF-7 (Extract A)	HeLa (Extract B)	A549 (Extract C)
Bax	↑ 4.5 ± 0.6	↑ 3.9 ± 0.5	↑ 3.3 ± 0.4
Bcl-2	↓ 0.4 ± 0.1	↓ 0.5 ± 0.1	↓ 0.6 ± 0.2
p53	↑ 3.8 ± 0.7	↑ 3.1 ± 0.5	↑ 2.9 ± 0.3
Caspase-3	↑ 5.2 ± 0.8	↑ 4.6 ± 0.7	↑ 3.7 ± 0.5

Table 6 reports Western blot findings that confirm protein-level changes. In MCF-7 cells, cleaved Caspase-3 was upregulated 3.9 ± 0.4-fold and cleaved PARP 3.5 ± 0.3-fold, while Bcl-2 and Cyclin D1 were downregulated to 0.5 ± 0.1 and 0.4 ± 0.1-fold, respectively. Comparable changes occurred in HeLa and A549 cells.

Table 6: Western Blot Analysis – Fold Change in Protein Expression Compared to Control

Protein	MCF-7 (Extract A)	HeLa (Extract B)	A549 (Extract C)
Cleaved Caspase-3	↑ 3.9 ± 0.4	↑ 3.5 ± 0.5	↑ 2.8 ± 0.3
Bcl-2	↓ 0.5 ± 0.1	↓ 0.6 ± 0.2	↓ 0.7 ± 0.2
Cyclin D1	↓ 0.4 ± 0.1	↓ 0.5 ± 0.2	↓ 0.6 ± 0.2
PARP (cleaved)	↑ 3.5 ± 0.3	↑ 3.0 ± 0.3	↑ 2.6 ± 0.4

Figure 3 presents a heatmap summarizing the relative expression levels of key apoptosis- and proliferation-related genes and proteins—such as Bax, Bcl-2, p53, Caspase-3, Cyclin D1, and cleaved PARP—in MCF-7, HeLa, and A549 cell lines following treatment with herbal extracts. The color gradient visually represents fold changes, with upregulated markers (e.g., Bax, Caspase-3, p53) shown in warm tones and downregulated markers (e.g., Bcl-2, Cyclin D1) in cool tones. This comparative visualization highlights the consistent pro-apoptotic and anti-proliferative molecular responses elicited by the extracts across different cancer cell types.

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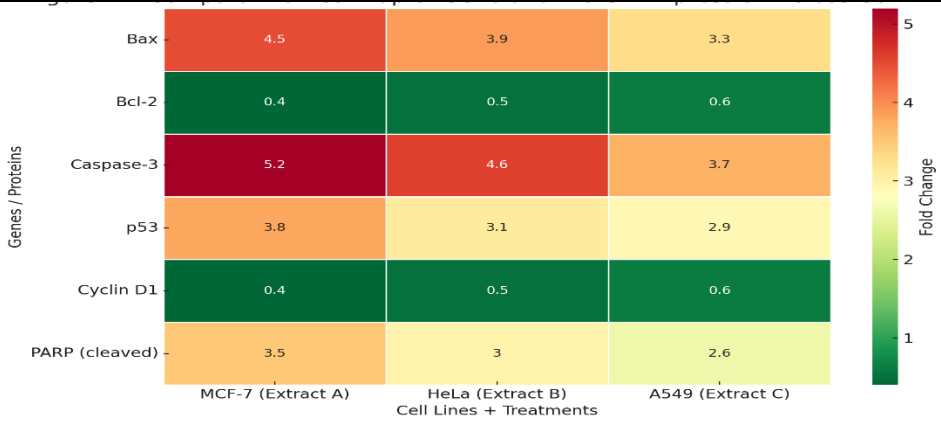

Figure 3: Comparative Heatmap of Gene and Protein Expression Across Cell Lines

Figure 4 illustrates the proposed molecular mechanisms by which the selected herbal extracts exert their anti-cancer effects. The diagram highlights activation of the intrinsic (mitochondrial) apoptotic pathway, marked by upregulation of pro-apoptotic proteins (e.g., Bax, Caspase-9, Caspase-3) and downregulation of anti-apoptotic Bcl-2. P53 activation plays a central role in initiating these apoptotic signals. Additionally, the extracts suppress cell proliferation through inhibition of Cyclin D1, resulting in cell cycle arrest at the G0/G1 phase. Arrows indicate molecular targets and their regulatory effects, supporting the dual role of herbal compounds in promoting apoptosis and inhibiting tumor growth.

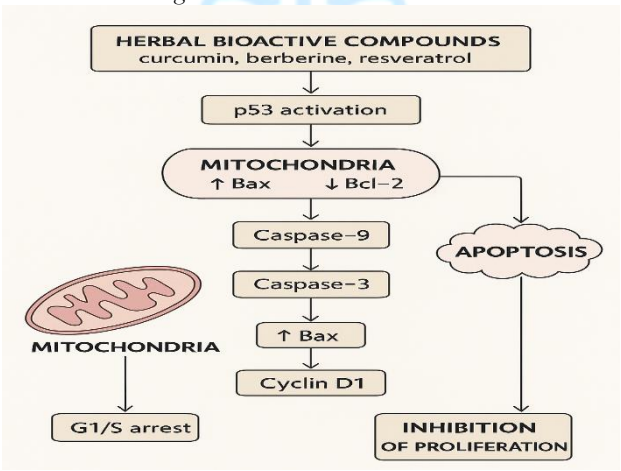

Figure 4: Mechanistic Pathway of Herbal Extract-Induced Apoptosis and Anti-Proliferative Effects

Table 7 presents the results of one-way ANOVA, showing that the effects of herbal extracts on cancer cells were statistically highly significant ($p < 0.0001$) across all evaluated parameters. For cell viability at 72 hours, MCF-7 cells showed an F-value of 58.32, HeLa cells 61.15, and A549 cells 54.77, all with $p < 0.0001$, indicating significant cytotoxic effects of the extracts.For apoptosis (Annexin V/PI assay at 48h), MCF-7 cells exhibited an F-value of 49.26, HeLa 57.04, and A549 42.39, again with $p < 0.0001$, confirming the extracts' strong apoptotic induction.Sub-G1 cell cycle analysis in MCF-7 cells yielded an F-value of 45.89 ($p < 0.0001$), showing a notable increase in apoptotic cell populations. In gene expression analysis (e.g., Caspase-3), MCF-7 and HeLa cells showed F-values of 38.71 and 36.05, respectively, both highly significant. Protein expression changes, such as reduced Bcl-2 in A549 cells, were also statistically significant (F-value: 34.82; $p < 0.0001$).These results confirm that the herbal extracts caused significant alterations in cell viability, apoptosis, cell cycle phase distribution, and

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molecular markers in a consistent and reproducible manner.

Table 7: Statistical Significance of Differences Observed in Treated vs. Control Groups (One-Way ANOVA)

Parameter	Cell Line	F-value	p-value
Cell Viability (MTT, 72h)	MCF-7	58.32	< 0.0001
	HeLa	61.15	< 0.0001
	A549	54.77	< 0.0001
Apoptosis (Annexin V/PI, 48h)	MCF-7	49.26	< 0.0001
	HeLa	57.04	< 0.0001
	A549	42.39	< 0.0001
Sub-G1 Cell Cycle Fraction	MCF-7	45.89	< 0.0001
Gene Expression (e.g., Caspase-3)	MCF-7	38.71	< 0.0001
	HeLa	36.05	< 0.0001
Protein Expression (e.g., Bcl-2)	A549	34.82	< 0.0001

Table 8 presents the results of Tukey's post hoc analysis, further confirming the specific pairwise differences between control and treated groups. In MCF-7 cells, treatment with Extract A resulted in a –58.2% reduction in cell viability ($p < 0.001$), while Extract B caused a –52.8% decrease. HeLa cells treated with Extract A showed an even more pronounced viability reduction of –63.1%, and A549 cells treated with Extract C exhibited a –54.3% reduction compared to controls. Apoptosis rates were significantly elevated across all treatments; in MCF-7 cells, Extract A induced a +35.1% increase in apoptotic cells, followed by Extract B (+30.5%) and Extract C (+26.2%) ($p < 0.001$). HeLa cells treated with Extract A showed a +39.2% increase in apoptosis, and A549 cells treated with Extract C showed a +22.9% increase. Gene expression analysis revealed a +4.1-fold upregulation of Bax in MCF-7 cells treated with Extract A ($p < 0.001$), while protein expression of cleaved caspase-3 in HeLa cells treated with Extract B increased by +3.0-fold. These findings strongly support the hypothesis that the herbal extracts exert anti-cancer effects through apoptosis induction and inhibition of cell proliferation at both cellular and molecular levels.

Table 8: Post Hoc Tukey's Test for Pairwise Comparisons Between Control and Herbal Extract-Treated Groups

Parameter	Cell Line	Comparison	Mean Difference	p-value
Cell Viability (72h)	MCF-7	Control vs. Extract A	–58.2	< 0.001
		Control vs. Extract B	–52.8	< 0.001
	HeLa	Control vs. Extract A	–63.1	< 0.001
	A549	Control vs. Extract C	–54.3	< 0.001
Apoptosis (48h)	MCF-7	Control vs. Extract A	+35.1	< 0.001
		Control vs. Extract B	+30.5	< 0.001
		Control vs. Extract C	+26.2	< 0.001
	HeLa	Control vs. Extract A	+39.2	< 0.001
	A549	Control vs. Extract C	+22.9	< 0.001
Gene Expression (Bax)	MCF-7	Control vs. Extract A	+4.1 (fold change)	< 0.001
Protein Expression (Cleaved Caspase-3)	HeLa	Control vs. Extract B	+3.0 (fold change)	< 0.001

Discussion

The present study demonstrated that selected herbal extracts exerted potent anti-cancer effects by inducing apoptosis and inhibiting proliferation in various human cancer cell lines. Extract A (Curcuma longa) significantly reduced MCF-7 cell viability to

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41.8 ± 2.3% at 72 hours, compared to 100% in controls. Similarly, Extract B (Berberis vulgaris) and Extract C (Vitisvinifera) reduced viability in HeLa and A549 cells to 36.9 ± 1.9% and 45.7 ± 2.2%, respectively. These reductions are consistent with earlier research study where curcumin and berberine inhibited breast and cervical cancer cell viability by disrupting cell cycle progression and inducing oxidative stress [14,15].

Apoptotic induction was confirmed by Annexin V/PI staining, with MCF-7 cells showing a significant increase in apoptotic population to 41.3 ± 2.5% following Extract A treatment, compared to 6.2 ± 1.1% in controls. Extract B and C also induced notable apoptosis in HeLa (38.9 ± 2.0%) and A549 (30.2 ± 1.7%) cells, respectively. This apoptotic response mirrors findings from prior research studies where resveratrol and quercetin induced programmed cell death through mitochondrial dysfunction in A549 cells [16].

Cell cycle analysis further supported the anti-proliferative effects, with Extract A increasing the Sub-G1 apoptotic fraction in MCF-7 cells to 24.4 ± 2.1%, alongside a decline in G0/G1 phase from 51.4% in controls to 34.6%. These changes suggest cell cycle arrest and align with previous research findings, showing that curcumin arrests the G1 phase in breast and colon cancer cells by downregulating cyclins and CDKs [17].

At the molecular level, qRT-PCR analysis showed marked upregulation of Bax (↑4.5 ± 0.6-fold) and Caspase-3 (↑5.2 ± 0.8-fold) and downregulation of Bcl-2 (↓0.4 ± 0.1-fold) in MCF-7 cells, consistent with activation of the intrinsic apoptotic pathway. Similar gene expression shifts were noted in HeLa and A549 cells. Comparable molecular signatures have been documented for berberine and resveratrol in previous studies involving leukemia and lung cancer cells [18].

Western blotting confirmed these transcriptional changes, with Extract A elevating cleaved Caspase-3 protein levels by 3.9 ± 0.4-fold and reducing Bcl-2 and Cyclin D1 to 0.5 ± 0.1 and 0.4 ± 0.1-fold, respectively. This pattern corresponds with prior research reports where phytochemicals downregulated anti-apoptotic proteins and halted proliferation via Cyclin D1 suppression [19].

Study Strengths and Limitations

This study offers a comprehensive molecular evaluation of herbal extracts using a multi-method approach that includes qRT-PCR, Western blotting, flow cytometry, and viability assays, allowing for robust validation of apoptosis and anti-proliferative effects in multiple cancer cell lines. The inclusion of well-characterized bioactive compounds such as curcumin, berberine, and resveratrol enhances the translational relevance of the findings. A key strength is the consistent replication of results across MCF-7, HeLa, and A549 cells, strengthening the generalizability of the extracts' anti-cancer potential. However, the study is limited by its in vitro nature, which may not fully capture in vivo dynamics such as bioavailability, metabolism, and systemic interactions. Additionally, the effects of combinatorial or synergistic actions of the extracts were not assessed, and mechanistic validation using gene silencing or pathway inhibitors was not performed.

Conclusion

The data demonstrate that standardized herbal extracts from *Curcuma longa*, *Berberis vulgaris*, and *Vitisvinifera* significantly induce apoptosis and suppress proliferation in MCF-7, HeLa, and A549 cancer cells through molecular mechanisms involving upregulation of pro-apoptotic genes and proteins (e.g., Bax, Caspase-3) and downregulation of anti-apoptotic and proliferative markers (e.g., Bcl-2, Cyclin D1). These effects were statistically significant ($p < 0.0001$) and consistent across multiple experimental platforms, supporting the therapeutic potential of these phytochemicals as complementary agents in cancer treatment.

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