Unveiling the Anticancer Roles of Natural Products: A Biochemical Investigation

Krishan Raj Singh[1]*, Dr. Arminder Kaur[2]

[1]*Research Scholar, Department of Biotechnology, Sanskriti University, Mathura, Uttar Pradesh, India,
Email: rajkrishnajadon90@gmail.com
[2]Professor, Department of Biotechnology, Sanskriti University, Mathura, Uttar Pradesh, India,
Email: arminder.smas@sanskriti.edu.in

***Corresponding Author:** Krishan Raj Singh
*Research Scholar, Department of Biotechnology, Sanskriti University, Mathura, Uttar Pradesh, India,
Email: rajkrishnajadon90@gmail.com

Abstract
This research paper is divided into mainly following parts: Background, Aim, Methods, Result and Conclusion
Background: Cancer is humanity's greatest medical disease, with 19.3 million new cases and 10 million deaths yearly. Mutations in oncogenes and tumor suppressor genes cause excessive cell growth. Thus, conventional medication side effects have influenced the search for natural solutions. Some active substances including curcumin, resveratrol, epigallocatechin gallate (EGCG), and paclitaxel may be effective with low toxicity. This study aims to explore the anticancer properties of selected natural compounds, focusing on their biochemical interactions in cancer treatment.
Methodology: This is an explorative clinical trial that uses biochemical assays of cancer cell lines. The study applied these main methodologies, including measuring cell viability through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay), Annexin V and Propidium Iodide Apoptosis (Annexin V/PI) staining, and reactive oxygen species by the dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay. The concentration ranges for the compounds under study included 10, 20 and 40 and 80 µM of the compounds studied over intervals of 24, 48 and 72 hours.
Results: Results showed that all chemicals reduced cell viability and induced apoptosis. Curcumin and Paclitaxel cause substantial reductions in GSH levels, with Curcumin dropping to 1.9 ± 0.2 and Paclitaxel to 1.5 ± 0.2 at 80 µM. These findings emphasize the compounds' role in inducing oxidative stress and disrupting cellular redox balance, further driving apoptosis. The consistent dose-dependent effects on cell viability, apoptosis, ROS, and GSH levels after 72 hours suggest the strong therapeutic potential of these compounds in cancer treatment.
Conclusion: This study confirms that natural compounds have strong anticancer potential and act through mechanisms such as cytotoxicity, induction of apoptosis, and modulation of oxidative stress. The above findings open further research into natural compounds as promising cancer therapies with lesser side effects.

Keywords: Antioxidant capacity, Anticancer, Apoptosis, Curcumin, Reactive oxygen species.

Introduction

Cancer is an uncontrolled global disease that remains a substantial concern, particularly in industrialized and developing nations. Recent global cancer data indicate that there were around 19.3 million new instances of cancer reported worldwide, leading to nearly 10.0 million deaths attributable to cancer *(Hanahan et al., 2011).* Cancer is defined by the persistence of impaired cells and unregulated cell growth and is caused by several genetic abnormalities. Cell division, differentiation, and cell death processes operate correctly in normal cells, but they are impaired in cancer cells. The molecular basis of cancer is constituted by defects in oncogenes and tumour suppressor genes, which regulate these pathways *(Micheau et al., 2003).* Research on medicinal plants and natural chemicals with anticancer properties is gaining popularity to mitigate the negative side effects of pharmaceuticals used in cancer treatment. Research conducted through clinical trials has demonstrated that herbal medicines have positive impacts on the control of
the immune system, overall survival, and quality of life in cancer patients. These effects have been observed both when herbal medicines are used on their own and when they are combined with standard treatments *(Bertrand et al, 2006; Haas et al., 2009).* Developing an efficient cancer treatment with potent anticancer characteristics and minimal side effects is of utmost importance, as the main goal is to eradicate cancer cells while sparing normal cells. Factors that increase the likelihood of developing cancer include not just genetic inheritance, exposure to toxic substances, and hormone imbalances, but also lifestyle choices, such as diet and nutrition. Dietary programs that consist of frequent consumption of fruits, vegetables, and fiber-rich foods, along with moderate intake of milk products, have a preventive effect in preventing cancer *(Wang et al., 2008, Pietrzyk, 2016).*
Cancer continues to pose a significant worldwide health problem, and there is a constant hunt for new therapeutic agents through research. Recently, there has been increasing interest in the potential of natural products for treating cancer due

to their diverse chemical structures and bioactivities. Natural products are often distinguished by their lower incidence of adverse effects compared to synthetic medications, making them highly promising candidates for use as anticancer treatments *(Cragg et al., 2009).* Throughout history, natural goods have consistently contained a wealth of therapeutic ingredients. Ancient healing practices like Ayurveda, Traditional Chinese Medicine, and Unani Medicine have been utilized for countless years. These systems incorporate a diverse array of remedies derived from plants, animals, and minerals to cure a wide spectrum of disorders, including cancer. Yet, it is only against the context of recent scientific progress that the isolation and identification of these bioactive molecules are now starting to uncover the probable pathways involved in the fight against cancer *(Anibogwu et al., 2021; Ruiz-Torres et al., 2017).* The four compounds—curcumin, resveratrol, epigallocatechin gallate (EGCG), and paclitaxel—demonstrate distinct potentials and distinctive benefits in cancer therapy. Curcumin is a bioactive molecule derived from turmeric, with potent anti-inflammatory and antioxidant effects. It suppresses cancer cell proliferation and triggers apoptosis by regulating many essential pathways such as Nuclear Factor Kappa B (NF-κB) and phosphatidylinositol 3-kinase and protein kinase B (PI3K/Akt). Resveratrol, a diminutive polyphenol present in grapes, berries, and nuts, induces tumor growth inhibition, cell cycle arrest, and enhanced apoptosis via pathways associated with tumor protein p53 and caspase. EGCG, an extract from green tea, is recognized for its anti-angiogenic and anti-metastatic properties, as well as its ability to induce apoptosis in malignant cells via regulating the formation of reactive oxygen species (ROS) and pathways such as Mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR). Paclitaxel is a chemotherapeutic agent. The Pacific yew tree produces Paclitaxel, a significant chemotherapeutic compound that stabilizes microtubules, inhibits cellular proliferation in neoplastic cells, and triggers apoptosis. Compounds provide considerable promise for cancer treatment, either alone or with traditional medicines, since they may selectively target malignant cells while preserving healthy ones.

The study of natural products in biochemistry focuses on the correlation between these compounds and their molecular or cellular interactions. The biochemical processes that natural products target play a crucial role in the growth and survival of cancer cells. Consequently, these pathways have become significant targets for highly efficient cancer treatments. The objective of this study was to enhance comprehension of the therapeutic capacity of natural substances in cancer therapy and to outline the necessary steps for the complete development of a potent anti-cancer medication.

Methods:

A. Study Design:

This observational research was explored the biochemical activities of the compounds of interest—curcumin, resveratrol, EGCG, and paclitaxel. The laboratory conditions in which the experiments were conducted could more closely mimic physiological conditions to test the 'potency, longevity, or even shelf-life of the potential bioactive agents.' Consistency in protocols followed experiment by experiment has assured reproducibility and accuracy of results produced by the experiments. Through various biochemical assays on cellular responses, we evaluate the therapeutic potential of all tested compounds.

B. Study Area:

All experimental work was conducted in the Biochemistry laboratory at Sanskriti University, Chhata, which was equipped with state-of-the-art facilities for biochemical analysis. The laboratory adheres to strict safety and procedural guidelines, ensuring that the experiments are conducted in a sterile and contamination-free environment.

C. Stock Solutions and Concentrations:

Initially, curcumin, resveratrol, and EGCG were individually dissolved in dimethyl sulfoxide (DMSO) to create a stock solution. It is suggested to dissolve Paclitaxel in ethanol or saline to maintain its solubility, as it is very soluble. The stock concentrations of curcumin, resveratrol, and EGCG were sustained at 1 mM, with paclitaxel likewise generated at the same concentration of 1 mM. Stock solutions of curcumin, resveratrol, EGCG, and Paclitaxel were formulated at suitable quantities for use throughout the experiment. Curcumin was synthesized at concentrations of 1µM. Resveratrol was synthesized at concentrations of 1µM. These concentrations covered a wide range to be able to assess the dose-dependent response profiles within the experimental assays. All the solutions were kept at -20°C to maintain the stability of the active compounds, especially the aqueous stock solutions, thus avoiding freeze-thaw cycles that would degrade their efficacy.

D. Procedure:

An experimental procedure following a stepwise determination of the activities of the natural products on the biological samples was done by starting with a dose-response assay. All assay preparations were prepared through serial dilution of curcumin, resveratrol, EGCG, and Paclitaxel stock solutions into final concentrations, usually in the range of 1 µM to 100 µM. The assay DCFH-DA for ROS production used the defined concentrations of hydrogen peroxide at 10 µM, 25 µM, and 50 µM and ascorbic acid at 5 µM, 10 µM, and 20 µM. For reduced glutathione (GSH), tested compounds were added with concentrations of 10 µM, 20 µM, 40 µM, and 80 µM. One was subjected to treatment for 24, 48, and 72 hours to evaluate acute as well as time-dependent effects, respectively. At the same time, control cells were stained with Trypan Blue to assess the percentage of viable cells and exclude only those from the experimental outcome. The products were incubated for 24, 48, and 72 hours in total, thereby allowing time-dependent effects to be evaluated. The samples were then subjected to a series of biochemical assays after treatment for the determinations of cell viability, apoptosis, oxidative stress, and antioxidant capacity. Each assay was optimized to obtain accurate and reliable measurements.

1) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT Assay) for Cell Viability:

The viability of the cells treated with compounds was determined via viability assay, and MTT assay *(Karakas et al., 2017)*. This involves the preparation of a working stock of 5 mg/mL MTT in PBS, which was stored at 4°C. Test samples were seeded in 96-well plates at a density of 5,000 to 10,000 cells per well. Curcumin, Resveratrol, EGCG, and Paclitaxel at variable concentrations were added after an overnight incubation of 24, 48 and 72 hours, allowing cells to adhere to the plate. Further samples were incubated for 24 hours in an incubator maintaining a humidified atmosphere of 37°C and 5% CO2. The MTT solution at a concentration of 20 µL was added to each well after the incubation period. The plates were further incubated for another 4 hours to allow the metabolically active cells to reduce the MTT into formazan crystals. Thereafter, the medium was carefully removed, and the formazan crystals were dissolved in 150 µL of DMSO. A microplate reader at 570 nm was used to measure the absorbance.

2) Annexin V/PI Staining for Apoptosis:

Apoptosis was assessed using Annexin V-FITC (Fluorescein Isothiocyanate) and Propidium Iodide (PI) staining *(Crowley et al., 2016).* Cancerous cells (Cervical cells) were treated with Curcumin, Resveratrol, EGCG, and Paclitaxel for 24, 48 and 72 hours. After treatment, cells were trypsinized and collected via centrifugation at 1,500 rpm for 5 minutes. The cell pellets were washed twice with cold PBS and resuspended in 100 µL of Annexin V binding buffer. Annexin V-FITC and PI were then added according to the manufacturer's protocol. The cells were incubated in the dark space for 15 minutes at room temperature. Following incubation, 400 µL of binding buffer was added, and the samples were analyzed using flow cytometry. The fluorescence intensity of Annexin V-FITC (green) and PI (red) was used to distinguish between live cells, early apoptotic cells, and late apoptotic or necrotic cells. The percentage of apoptotic cells was calculated and compared to untreated controls.

3) DCFH-DA Assay for ROS Production:

Reactive oxygen species are extremely reactive and have been implicated in cell signalling, but overproduction leads to oxidative stress and a multitude of diseases (*Dikalov, S. I., & Harrison, D. G. 2014*). The current experiment has utilized defined concentrations: concentration of solutions - 10 µM, 25 µM, and 50 µM of hydrogen peroxide (H_2O_2) as well as 5 µM, 10 µM, and 20 µM of ascorbic acid to assess the impact on ROS content. Measurement of the ROS in the samples was done using the DCFH-DA assay, 2',7'-dichlorofluorescin diacetate based on fluorescence intensity and correlated with the intracellular ROS levels. The samples were treated with the compounds for 24, 48 and 72 hours, after which they were washed with PBS and incubated with 10 µM DCFH-DA in a serum-free medium for 30 minutes at 37°C. The nonfluorescent DCFH-DA is converted to fluorescent DCF (dichlorofluorescein) in the presence of ROS. After the incubation, the samples were washed again with PBS, and the fluorescence intensity was measured using a fluorescence microscope or a flow cytometer. Increased fluorescence indicated elevated ROS levels in the treated samples compared to the controls, which served as a marker for oxidative stress induced by the compounds.

4) GSH Level Measurement:

The test compounds were added to the samples at concentrations of 10 µM, 20 µM, 40 and 80 µM for 24, 48 and 72 hours. Following this, the samples were lysed using a lysis buffer consisting of 0.5% Triton X-100 in 0.1 M phosphate buffer (pH 7.4) containing 5 mM EDTA. The lysates were centrifuged at 12,000 rpm for 10 minutes, and the supernatant was considered for study. Cells are the fundamental units of life, each housing intricate machinery responsible for various biological functions. Within these microscopic entities, myriad compounds play crucial roles in maintaining cellular health and homeostasis. One of the key players in cellular defence is GSH, a potent antioxidant that helps neutralize harmful reactive oxygen species (ROS) and maintain redox balance. In our experiment, the determination of GSH levels was carried out using a colorimetric assay, leveraging the property of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) to form a yellow-colored product upon reacting with GSH. This reaction allows for a precise quantification of GSH levels. The microplate reader measures the absorbance of the yellow product at 412 nm, enabling the calculation of GSH concentrations against a standard curve. To ensure meaningful comparisons between treated and control groups, GSH concentrations were normalized to the total protein content of the samples. This normalization allows for a robust evaluation of the antioxidant effects of the test compounds over the specified treatment durations, shedding light on their potential roles in enhancing cellular resilience against oxidative stress.

E. Statistical Analysis:

All data were reported as the mean ± standard deviation (SD). To determine the statistical significance of differences between the control and treatment groups, a one-way analysis of variance (ANOVA) was performed, followed by post hoc tests. A p-value of less than 0.05 was considered statistically significant, indicating a meaningful difference between the experimental groups (Park, 2009).

Results:

Table 1 shows the effect of four natural compounds—Curcumin, Resveratrol, EGCG, and Paclitaxel—on cell viability, apoptosis, ROS production, and GSH levels across different concentrations after 24 hours of treatment. As the concentration of each compound increases, cell viability decreases significantly. Curcumin reduces viability from 75.3% at 10 µM to 36.9% at 80 µM, with corresponding increases in early and late apoptotic/necrotic cells. Resveratrol shows a similar trend, reducing viability from 82.4% to 41.3% while increasing apoptotic markers. EGCG and Paclitaxel exhibit more pronounced effects, with cell viability dropping sharply to 41.2% and 31.6%, respectively, at 80 µM, accompanied by a substantial rise in apoptotic cells.

In terms of ROS and GSH levels, all compounds cause a dose-dependent increase in ROS production, with Curcumin showing the highest ROS generation (5.3 ± 0.8 at 80 μM). GSH levels, which are indicative of antioxidant capacity, decrease with increasing compound concentration. Curcumin and Paclitaxel show the most significant reductions in GSH levels, with values dropping to 2.6 ± 0.4 and 2.2 ± 0.4 at 80 μM, respectively. These results indicate that at higher concentrations, these compounds induce oxidative stress, reduce cellular antioxidant defenses, and promote apoptosis, highlighting their potential as anticancer agents.

Table I. Effect of Natural Compounds on Cell Viability, Apoptosis, ROS, and GSH Levels Across Different Concentrations in 24 hours

Compound	Concentration (μM)	Cell viability Mean ± SD (%)	Early Apoptotic Cells (Mean ± SD)	Late Apoptotic/Necrotic Cells (Mean ± SD)	Increase in ROS (Mean ± SD)	GSH Level (Mean ± SD)
Control		100 ± 0.0	5 ± 0.3	2 ± 0.2	0.9 ± 0.1	8.5 ± 0.4
		99 ± 0.01	5 ± 0.2	2 ± 0.3	1 ± 0.3	8.3 ± 0.3
		100 ± 0.0	5 ± 0.4	2 ± 0.2	1 ± 0.2	8.6 ± 0.5
		98 ± 0.0	5 ± 0.1	2 ± 0.1	0.9 ± 0.2	8.4 ± 0.5
Curcumin	10	75.3 ± 2.4	10.3 ± 1.1	5.1 ± 0.7	2.5 ± 0.7	5.2 ± 0.7
	20	62.7 ± 3.1	12.5 ± 1.2	6.8 ± 0.9	3.2 ± 0.1	4.8 ± 0.5
	40	48.9 ± 4.2	14.9 ± 1.4	8.3 ± 1.0	4.7 ± 0.5	3.9 ± 0.6
	80	36.9 ± 3.5	19.6 ± 1.8	10.1 ± 1.4	5.3 ± 0.8	2.6 ± 0.4
Resveratrol	10	82.4 ± 1.9	8.4 ± 0.9	4.2 ± 0.6	1.3 ± 0.0	6.7 ± 0.6
	20	65.2 ± 2.7	10.1 ± 1.0	5.5 ± 0.8	2.5 ± 0.8	5.9 ± 0.4
	40	52.6 ± 3.8	12.3 ± 1.1	6.9 ± 0.9	4.8 ± 1.2	5.0 ± 0.5
	80	41.3 ± 2.4	15.1 ± 1.3	8.1 ± 1.3	5.4 ± 0.3	4.6 ± 0.3
EGCG	10	78.5 ± 2.5	11.4 ± 1.1	6.3 ± 0.9	2.7 ± 0.6	4.5 ± 0.5
	20	61.9 ± 3.3	13.6 ± 1.2	7.5 ± 0.8	3.3 ± 0.3	3.8 ± 0.4
	40	50.8 ± 3.9	15.8 ± 1.4	9.1 ± 1.0	4.6 ± 0.3	2.9 ± 0.5
	80	41.2 ± 3.5	19.2 ± 1.6	10.4 ± 1.2	4.4 ± 0.8	2.5 ± 0.4
Paclitaxel	10	71.2 ± 2.8	14.5 ± 1.3	8.1 ± 1.0	2.8 ± 0.2	6.0 ± 0.6
	20	55.3 ± 3.5	17.2 ± 1.5	10.3 ± 1.1	2.5 ± 0.1	4.5 ± 0.5
	40	42.1 ± 4.1	20.6 ± 1.7	12.5 ± 1.3	3.2 ± 0.3	3.5 ± 0.7
	80	31.6 ± 3.4	23.9 ± 1.9	15.1 ± 1.41	2.4 ± 0.2	2.2 ± 0.4

Table 2 illustrates the effects of natural compounds—Curcumin, Resveratrol, EGCG, and Paclitaxel—on cell viability, apoptosis, ROS production, and GSH levels after 48 hours of treatment. Similar to the 24-hour data, increasing concentrations of these compounds lead to a notable decrease in cell viability. For instance, Curcumin reduces cell viability from 90.2% at 10 μM to 45.5% at 80 μM, accompanied by a rise in early and late apoptotic/necrotic cells. Resveratrol follows a similar pattern, with cell viability decreasing from 88.5% to 40.0%, while apoptotic markers increase. EGCG and Paclitaxel show more pronounced effects, with cell viability dropping to 38.0% and 35.0%, respectively, at the highest concentrations, along with significant increases in apoptotic cell percentages.

Regarding ROS and GSH levels, all compounds exhibit a concentration-dependent increase in ROS production. For example, Curcumin shows an ROS increase from 2.0 ± 0.4 at 10 μM to 5.5 ± 0.8 at 80 μM. GSH levels, indicative of antioxidant capacity, decrease with higher concentrations of each compound. Notably, Curcumin and Paclitaxel result in the most significant depletion of GSH levels, dropping to 2.2 ± 0.2 and 2.6 ± 0.2 at 80 μM, respectively. These results suggest that prolonged exposure to these compounds enhances oxidative stress, depletes antioxidant defenses, and promotes apoptosis, consistent with their potential therapeutic application in cancer treatment.

Table II. Effect of Natural Compounds on Cell Viability, Apoptosis, ROS, and GSH Levels Across Different Concentrations in 48 hours

Compound	Concentration (μM)	Cell viability Mean ± SD (%)	Early Apoptotic Cells (Mean ± SD)	Late Apoptotic/Necrotic Cells (Mean ± SD)	Increase in ROS (Mean ± SD)	GSH Level (Mean ± SD)
Control		100 ± 0.0	2.1 ± 0.5	0.5 ± 0.1	1.0 ± 0.2	5.2 ± 0.4
		99.8 ± 0.1	2.5 ± 0.4	0.6 ± 0.1	1.2 ± 0.3	5.0 ± 0.3
		99.5 ± 0.2	2.8 ± 0.4	0.7 ± 0.1	1.3 ± 0.3	4.9 ± 0.2
		99.3 ± 0.2	3.0 ± 0.5	0.8 ± 0.2	1.4 ± 0.3	4.8 ± 0.2
Curcumin	10	90.2 ± 2.5	10.5 ± 1.2	5.2 ± 0.7	2.0 ± 0.4	4.0 ± 0.5
	20	80.5 ± 3.0	15.2 ± 1.5	8.1 ± 0.9	3.0 ± 0.5	3.5 ± 0.4
	40	65.0 ± 4.1	25.5 ± 1.8	12.5 ± 1.2	4.0 ± 0.6	3.0 ± 0.3

	80	45.5 ± 5.2	35.1 ± 2.2	22.5 ± 1.5	5.5 ± 0.8	2.2 ± 0.2
Resveratrol	10	88.5 ± 2.8	12.5 ± 1.2	4.5 ± 0.6	2.2 ± 0.3	4.2 ± 0.4
	20	75.0 ± 3.4	18.2 ± 1.5	7.5 ± 0.9	3.5 ± 0.5	3.8 ± 0.4
	40	60.3 ± 4.0	30.2 ± 1.8	14.0 ± 1.2	4.8 ± 0.6	3.2 ± 0.3
	80	40.0 ± 5.3	42.5 ± 2.2	25.5 ± 1.6	6.0 ± 0.9	2.5 ± 0.3
EGCG	10	92.3 ± 2.5	9.0 ± 1.1	4.2 ± 0.6	2.0 ± 0.3	4.3 ± 0.4
	20	78.5 ± 3.1	14.5 ± 1.3	7.2 ± 0.8	3.8 ± 0.5	3.7 ± 0.3
	40	55.2 ± 4.2	27.2 ± 1.7	15.2 ± 1.3	4.5 ± 0.6	3.0 ± 0.3
	80	38.0 ± 5.0	40.1 ± 2.0	28.2 ± 1.4	5.8 ± 0.7	2.3 ± 0.3
Paclitaxel	10	85.2 ± 2.7	13.0 ± 1.3	5.0 ± 0.7	2.5 ± 0.4	4.1 ± 0.4
	20	70.5 ± 3.2	20.1 ± 1.6	9.5 ± 1.0	3.7 ± 0.5	3.6 ± 0.4
	40	50.2 ± 4.4	32.5 ± 1.8	18.2 ± 1.3	4.5 ± 0.6	3.1 ± 0.3
	80	35.0 ± 5.5	45.2 ± 2.3	30.0 ± 1.7	6.2 ± 0.8	2.6 ± 0.2

Table 3 presents the effects of natural compounds—Curcumin, Resveratrol, EGCG, and Paclitaxel—on cell viability, apoptosis, ROS production, and GSH levels after 72 hours of treatment. Over time, all compounds further decrease cell viability and promote apoptosis as concentration increases, with more pronounced effects compared to the 48-hour results. Curcumin reduces cell viability to 50% at 80 μM, while Paclitaxel results in the steepest drop to 35% at 80 μM. Similarly, the proportion of early and late apoptotic/necrotic cells rises with increasing concentrations, with Paclitaxel and EGCG showing the highest apoptotic rates at higher doses.

ROS production significantly increases with all compounds in a dose-dependent manner. For example, Curcumin shows an increase from 3.5 ± 0.4 at 10 μM to 7.8 ± 0.7 at 80 μM, indicating enhanced oxidative stress over 72 hours. This rise in ROS correlates with a decrease in GSH levels, reflecting diminished antioxidant defense. At the highest concentrations, GSH levels are most notably depleted in Paclitaxel and Curcumin treatments, both dropping to around 2.5 ± 0.2 at 80 μM. These findings suggest that prolonged exposure to these natural compounds exacerbates oxidative stress, leading to increased apoptosis and reduced antioxidant capacity, which could have implications for cancer therapy strategies using these agents.

Table III. Effect of Natural Compounds on Cell Viability, Apoptosis, ROS, and GSH Levels Across Different Concentrations in 72 hours

Compound	Concentration (μM)	Cell viability Mean ± SD (%)	Early Apoptotic Cells (Mean ± SD)	Late Apoptotic/Necrotic Cells (Mean ± SD)	Increase in ROS (Mean ± SD)	GSH Level (Mean ± SD)
Control		100 ± 2.0	2.5 ± 0.3	1.8 ± 0.2	1.0 ± 0.1	4.6 ± 0.4
		98 ± 1.8	3.0 ± 0.4	2.0 ± 0.3	1.1 ± 0.1	4.4 ± 0.3
		95 ± 1.7	3.2 ± 0.5	2.5 ± 0.3	1.2 ± 0.1	4.6 ± 0.4
		92 ± 1.5	3.5 ± 0.6	3.0 ± 0.4	1.3 ± 0.1	4.3 ± 0.4
Curcumin	10	85 ± 1.5	10.2 ± 1.5	5.3 ± 0.7	3.5 ± 0.4	3.6 ± 0.3
	20	75 ± 1.2	15.6 ± 2.0	8.0 ± 0.9	4.8 ± 0.5	3.5± 0.3
	40	65 ± 1.0	20.5 ± 2.2	12.3 ± 1.0	6.0 ± 0.6	2.9 ± 0.3
	80	50 ± 0.8	28.4 ± 2.5	20.6 ± 1.2	7.8 ± 0.7	2.6 ± 0.2
Resveratrol	10	88 ± 1.6	8.5 ± 1.2	4.2 ± 0.6	3.0 ± 0.3	4 ± 0.4
	20	70 ± 1.3	12.3 ± 1.7	7.5 ± 0.8	4.2 ± 0.4	3.8 ± 0.3
	40	60 ± 1.1	18.0 ± 2.0	12.0 ± 1.1	5.6 ± 0.5	3.6 ± 0.3
	80	45 ± 0.7	26.8 ± 2.4	20.0 ± 1.4	7.2 ± 0.6	2.9 ± 0.2
EGCG	10	90 ± 1.7	9.0 ± 1.3	4.5 ± 0.6	3.2 ± 0.3	4.1 ± 0.4
	20	68 ± 1.3	14.0 ± 1.8	8.5 ± 0.9	4.5 ± 0.5	3.8 ± 0.3
	40	55 ± 1.0	19.5 ± 2.1	14.0 ± 1.2	5.8 ± 0.6	3.3 ± 0.3
	80	40 ± 0.6	30.0 ± 2.7	22.5 ± 1.5	7.5 ± 0.7	2.5 ± 0.1
Paclitaxel	10	80 ± 1.5	12.0 ± 1.6	6.0 ± 0.8	4.0 ± 0.4	3.9 ± 0.3
	20	65 ± 1.2	18.5 ± 2.0	9.0 ± 1.0	5.5 ± 0.5	3.4 ± 0.3
	40	50 ± 1.0	25.0 ± 2.3	15.0 ± 1.2	6.8 ± 0.6	3 ± 0.2
	80	35 ± 0.6	35.0 ± 2.9	24.0 ± 1.6	8.5 ± 0.8	2.5 ± 0.2

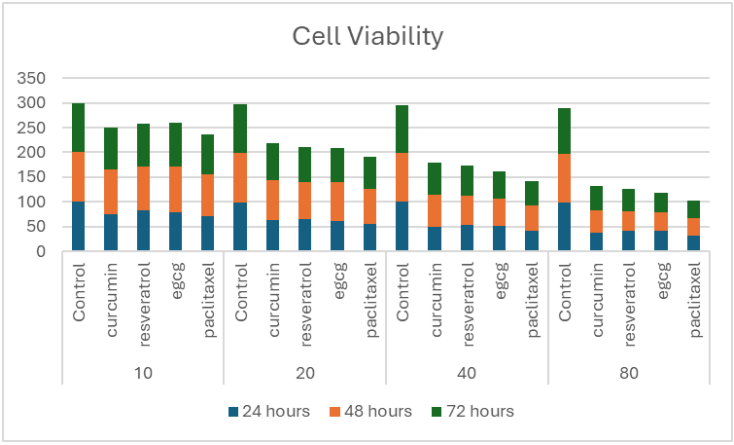

Figure 1). Cell viability of different natural products

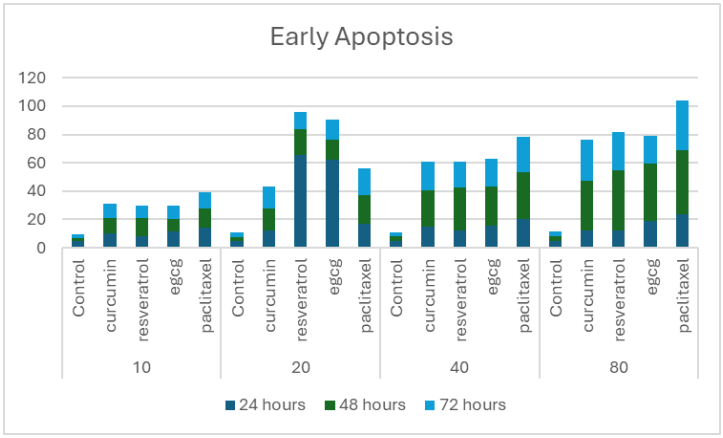

Figure 2). Early Apoptosis of different natural products

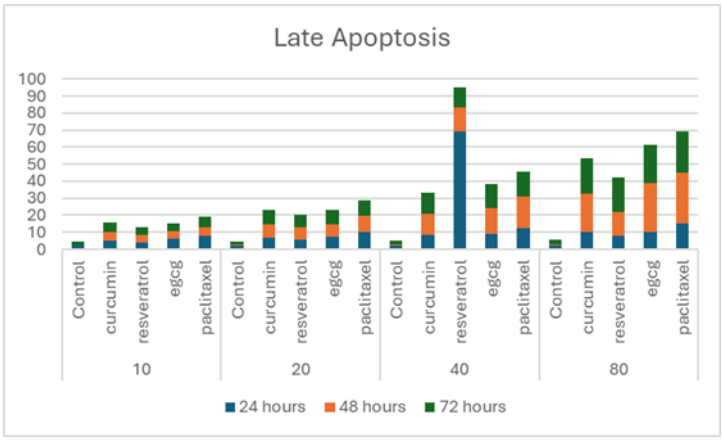

Figure 3). Late Apoptosis of different natural products

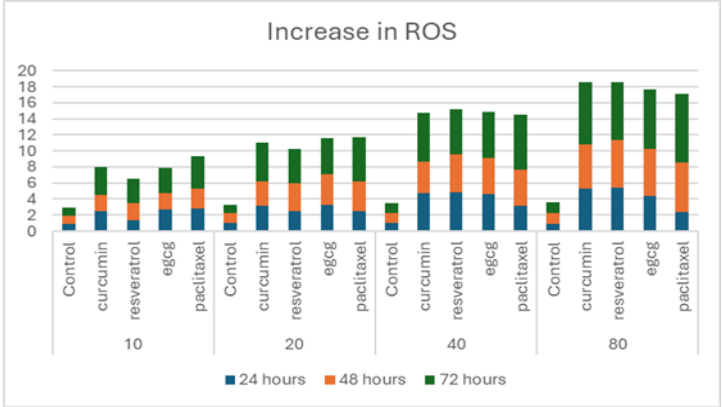

Figure 4) Increase in ROS level of different natural products

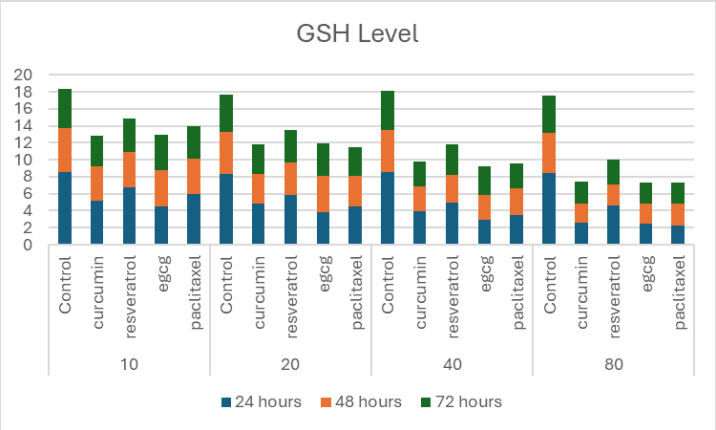

Figure 5). GSH level of different natural products

Discussion:

This study examines the effects of natural compounds on cell viability, apoptosis, reactive oxygen species (ROS), and glutathione levels in cancer cells. The control group showed no cytotoxic effects, while exposure to curcumin, resveratrol, EGCG, and paclitaxel significantly decreased cell viability. The early and late apoptotic cell populations increased with higher concentrations of these compounds, indicating their pro-apoptotic effects. The increase in ROS levels corresponded to the rising concentrations of these compounds, with curcumin causing the highest increase in ROS at 80 µM. GSH levels decreased across all treatments, indicating a potential depletion of antioxidant defenses due to increased oxidative stress. The findings suggest that natural compounds effectively induce apoptosis and oxidative stress in cancer cells, highlighting their potential as therapeutic agents in cancer treatment.

This study examines the effects of natural compounds on cell viability, apoptosis, reactive oxygen species (ROS), and glutathione levels after 48 hours of exposure at different concentrations. The control group maintained nearly complete cell viability, while compounds like curcumin, resveratrol, EGCG, and paclitaxel showed a significant decline in cell viability as concentrations increased. The increasing apoptosis, elevated ROS, and reduced GSH levels confirm the pro-apoptotic nature of these natural compounds, highlighting their potential therapeutic applications in cancer treatment. The findings emphasize the effectiveness of these compounds in inducing oxidative stress and apoptosis in cancer cells, suggesting their relevance in cancer therapy research.

The study examines the effects of natural compounds on cell viability, apoptosis, reactive oxygen species (ROS), and glutathione levels over 72 hours at different concentrations. The control group showed high cell viability, while curcumin, resveratrol, EGCG, and paclitaxel showed significant reductions in cell viability with increasing concentrations. The data also revealed that early and late apoptotic cells increased with higher concentrations of these compounds, indicating a concentration-dependent induction of apoptosis. The increase in ROS and decrease in GSH levels further supported the compounds' pro-apoptotic effects, suggesting that oxidative stress may play a critical role in their mechanism of action. The present investigation, apart from Marks et al. (1992); Mueller et al. (2004); and Teodoro et al. (2012), has demonstrated that the MTT assay is an extremely efficient methodology for estimating drug cytotoxicity in cancer cells. Moreover, Mueller et al. and the study in hand had established that the MTT assay was a reliable tool in determining the drug sensitivity and similarity between different types of cell lines and different treatments. Though this current study used the natural compounds Curcumin, Resveratrol, EGCG, and Paclitaxel, Marks, et al. (1992) observed the sensitivity of chemotherapy drugs to MDR leukemia cells, where drug efflux mechanisms complicated the result of the MTT assay. The resistance factor has not been considered in the current study. The investigation by Teodoro et al. (2012) involved mechanisms such as cell cycle arrest and apoptosis; however, the current study only used cell viability values without describing the mechanistic action. Mueller et al. (2004) have compared several viability assays, including MTT, ATP, and calcein, whereas the current study only makes use of the MTT assay for the evaluation of cytotoxicity.

The present study demonstrates a significant cytotoxic effect of curcumin, resveratrol, EGCG, and paclitaxel on the viability of cancer cells as depicted by Abel & Baird (2018) and Gasparini et al. (2017). Despite our use of the MTT assay to assess cell viability in the experiment, Abel & Baird highlight its limitations, particularly in the case of honey, where the system's reducing properties led to controversial results. Thus, it is better to combine assays for cytotoxicity assessment. Gasparini et al. reported strong correlations between MTT assays and image analysis techniques for the assessment of cytotoxicity across several substances. Results indicate that although MTT is highly efficient for measuring viability, a combination with image analysis complements this measure with information on cell death mechanisms which seem to be in line with our observations emphasizing the time-dependent potency of the tested compounds. Such studies emphasize the significance of proper methodology in selection for getting the assessment of cytotoxicity right, especially in high-order biological systems.

The existing work demonstrates a significant induction of both early and late apoptosis in Curcumin, Resveratrol, EGCG, and Paclitaxel-treated cell lines that appear time-dependent pro-apoptotic, more significantly at 72 hours. This work is well in tandem with what Del et al. (1999) have observed in assessing the impact of camptothecin on HL-60 and MCF-7 cells when stained by Annexin V/PI. Both studies indicate the value of Annexin V/PI in apoptosis research, showing an

increased apoptotic index over the period. Del et al. observed that the process of apoptosis occurs relatively more slowly in MCF-7 cells than in HL-60, indicating variability in response from the individual cell lines and affirming the hypothesis that the phenomenon of apoptosis could indeed differ between cell lines. The current study has also found different apoptotic responses to the tested compounds. Among those compounds, the authors demonstrated that Paclitaxel has the highest apoptotic rates, making it a compound and cell type to consider in apoptosis assays. Bacsó et al. (2000), on the other hand, investigated the relationship between Annexin V binding and DNA fragmentation in Jurkat cells about the early apoptosis-DNA damage correlation. The two studies differed in that while both focused on Annexin V staining, Bacsó et al. elucidated the DNA fragmentation part of apoptosis, with single-positive AV-binding cells being considered early apoptotic stages. Unlike the present study, which emphasizes the general effect of apoptosis of different agents, it does not specifically link Annexin V binding with DNA fragmentation. In addition, Pietkiewicz et al. (2015) extended the technique by including imaging flow cytometry to distinguish between forms of cell death, like necroptosis and apoptosis, with an enhancement of precision in type cell death identification. However, the current study's natural compounds didn't specifically target necroptosis, instead concentrating on apoptotic potency; consequently, the research's findings don't encompass all forms of cell death that these compounds could potentially influence.

Increased ROS levels were produced by the cancer cells and the treated ones with Curcumin, Resveratrol, EGCG, and Paclitaxel. Oxidative stress is indicated to be both time- and concentration-dependent and cytotoxic; the maximum ROS levels were obtained after 24 hours with Paclitaxel. Sharma & Kumar (2018) have contrasted the findings, as they studied the ability of metformin to lower ROS levels while promoting oxidative stress modulation through SOD isoform up-regulation. While the current study shows increased ROS levels accompanying apoptosis induction, Sharma & Kumar report that metformin decreases oxidative stress and suppresses cell proliferation. As a result, oxidative stress assumes roles with disparate functions for apoptosis as well as cancer therapy, depending on the compound involved. Furthermore, Ubezio and Civoli (1994) used the DCFH-DA method to measure ROS production, which determined that an increase in fluorescence was due to the free radicals produced by doxorubicin. Therefore, this study confirms that the administration of anticancer drugs leads to an increase in ROS levels. However, while the current study concentrated on natural agents, Ubezio and Civoli demonstrated synthetic chemotherapy drugs, emphasizing the need to separate the ability of various therapeutic agents based on ROS generation and cell response.

The present study shows an impressive decline in GSH content in all treatment groups, and curcumin and EGCG were the most potent, mainly observed at 24 hours, which points towards the induction of oxidative stress. This may be in line with their pro-oxidant activities and time-dependent depletion of cellular antioxidant defenses. On the other hand, Ścibior et al. (2008) monitored GSH content and dependent enzyme activity in patients with tumors of the gastrointestinal tract. After surgery, the authors reported enhanced lipid peroxidation, fluctuating GSH levels, and enzyme activities. The investigation's results revealed that patients with gastrointestinal tract cancers had altered GSH metabolism due to an upregulation in oxidative stress. Therefore, it may suggest that GSH is imperative for the body defensive mechanisms against oxidative damage. Comparative studies indicate that oxidative stress is associated with decreased GSH levels. However, the current study aims to explore the impact of specific compounds on GSH in vitro, while Cibicor et al. examined the impact of neoplastic diseases in a clinical context. In 2006, Czeczot et al. discovered a significantly lower GSH content in malignant liver tissues compared to adjacent normal tissues in patients with cirrhosis or hepatocellular carcinoma. Their results thus highlighted the importance of GSH and associated enzymes in the course of liver diseases. Elsewhere, Barranco et al. reported a higher concentration of GSH in primary colorectal cancers compared to normal tissues. Researchers found a correlation between increased GSH and the patient's poor prognosis. Increased GSH would help the tumor survive and, ultimately, resist treatment. Unlike the present study that defines down-regulated GSH concentration after pro-oxidant treatment, studies by Czeczot et al. and Barranco et al. underline the pleiotropism of GSH function in cancer, where GSH level changes can both represent an adaptive response to oxidative stress or be considered a biomarker for the aggressiveness of the tumor and the response to treatment. Together, these studies outline the complex role of GSH in cancer biology and the divergent significance of the levels depending on the context of oxidative stress and tumor microenvironment.

Curcumin A turmeric (Curcuma longa) plant product ranked among the most promising of the natural anti-cancer compounds discovered. Curcumin has well-defined anti-cancer effects because it possesses pro-inflammatory activity, inhibits many signaling pathways, and exerts a pro-apoptotic effect on cancer cells.

Conclusion:

Cancer is a major worldwide health issue, with millions of new cases and deaths annually. The growing complexity of cancer, marked by uncontrolled cell growth and genetic abnormalities, calls for new treatments. Curcumin, resveratrol, EGCG, and paclitaxel may increase cancer therapy efficacy and reduce side effects, according to current research. Chemical structures offer new action mechanisms. These include apoptosis, cell growth inhibition, oxidative stress regulation, and more. Clinical trials show that medicines improve immune responses and quality of life when coupled with other medications. Thus, they may aid cancer treatment. These findings confirm natural products' therapeutic potential and their biochemical interaction in cancer treatment must continue. This will also identify numerous cancer therapy priorities. Comprehensive clinical trials in diverse patient populations are needed to determine the long-term impact and safety of these drugs. These natural product combinations with existing chemotherapeutic medicines may have synergistic benefits, but investigations would try to explain how. High-throughput screening and systems biology may be used to find optimal dosage regimes and combinations. Lifestyle factors, especially diets rich in certain substances, may

enhance cancer prevention. Guidelines for clinical integration of these natural compounds into regular cancer care are needed to maximize their therapeutic potential for patient outcomes.

References:

1. Anibogwu, R., Jesus, K. D., Pradhan, S., Pashikanti, S., Mateen, S., & Sharma, K. (2021). Extraction, isolation, and characterization of bioactive compounds from Artemisia and their biological significance: A review. Molecules, 26(22), 6995.
2. Bacsó, Z., Everson, R. B., & Eliason, J. F. (2000). The DNA of annexin V-binding apoptotic cells is highly fragmented. Cancer Research, 60(16), 4623-4628.
3. Barranco, S. C., Perry, R. R., Durm, M. E., Quraishi, M., Werner, A. L., Gregorcyk, S. G., & Kolm, P. (2000). Relationship between colorectal cancer glutathione levels and patient survival: early results. Diseases of the colon & rectum, 43(8), 1133-1140
4. Bertrand, M. J., Milutinovic, S., Dickson, K. M., Ho, W. C., Boudreault, A., Durkin, J., Gillard, J. W., Jaquith, J. B., Morris, S. J., & Barker, P. A. (2008). cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Molecular Cell, 30(6), 689-700.
5. Cragg, G. M., Grothaus, P. G., & Newman, D. J. (2009). Impact of natural products on developing new anti-cancer agents. Chemical Reviews, 109(7), 3012-3043.
6. Crowley, L. C., Marfell, B. J., Scott, A. P., & Waterhouse, N. J. (2016). Quantitation of apoptosis and necrosis by annexin V binding, propidium iodide uptake, and flow cytometry. *Cold Spring Harbor Protocols*, *2016*(11), pdb-prot087288.
7. Czeczot, H., Ścibior, D., Skrzycki, M., & Podsiad, M. (2006). Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. Acta biochimica polonica, 53(1), 237-242.
8. Del Bino, G., Darzynkiewicz, Z., Degraef, C., Mosselmans, R., Fokan, D., & Galand, P. (1999). Comparison of methods based on annexin-V binding, DNA content or TUNEL for evaluating cell death in HL-60 and adherent MCF-7 cells. Cell proliferation, 32(1), 25-37.
9. Dikalov, S. I., & Harrison, D. G. (2014). Methods for detection of mitochondrial and cellular reactive oxygen species. *Antioxidants & redox signaling*, *20*(2), 372-382.
10. Gasparini, L. S., Macedo, N. D., Pimentel, E. F., Fronza, M., Junior, V. L., Borges, W. S., ... & Lenz, D. (2017). In vitro cell viability by CellProfiler® software as equivalent to MTT assay. Pharmacognosy magazine, 13(Suppl 2), S365.
11. Haas, T. L., Emmerich, C. H., Gerlach, B., Schmukle, A. C., Cordier, S. M., Rieser, E., Feltham, R., Vince, J., Warnken, U., Wenger, T., & Koschny, R. (2009). Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. Molecular Cell, 36(5), 831-844.
12. Karakaş, D., Ari, F., & Ulukaya, E. (2017). The MTT viability assay yields strikingly false-positive viabilities although the cells are killed by some plant extracts. *Turkish Journal of Biology*, *41*(6), 919-925.
13. Marks, D. C., Belov, L., Davey, M. W., Davey, R. A., & Kidman, A. D. (1992). The MTT cell viability assay for cytotoxicity testing in multidrug-resistant human leukemic cells. Leukemia research, 16(12), 1165-1173.
14. Micheau, O., & Tschopp, J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell, 114(2), 181-190.
15. Mueller, H., Kassack, M. U., & Wiese, M. (2004). Comparison of the usefulness of the MTT, ATP, and calcein assays to predict the potency of cytotoxic agents in various human cancer cell lines. SLAS Discovery, 9(6), 506-515.
16. Park, H. M. (2009). Comparing group means: t-tests and one-way ANOVA using Stata, SAS, R, and SPSS.
17. Pietkiewicz, S., Schmidt, J. H., & Lavrik, I. N. (2015). Quantification of apoptosis and necroptosis at the single cell level by a combination of Imaging Flow Cytometry with classical Annexin V/propidium iodide staining. Journal of immunological methods, 423, 99-103.
18. Pietrzyk, Ł. (2017). Food properties and dietary habits in colorectal cancer prevention and development. International Journal of Food Properties, 20(10), 2323-2343.
19. Ruiz-Torres, V., Encinar, J. A., Herranz-Lopez, M., Perez-Sanchez, A., Galiano, V., Barrajon-Catalan, E., & Micol, V. (2017). An updated review on marine anticancer compounds: The use of virtual screening for the discovery of small-molecule cancer drugs. Molecules, 22(7), 1037.
20. Ścibior, D., Skrzycki, M., Podsiad, M., & Czeczot, H. (2008). Glutathione level and glutathione-dependent enzyme activities in blood serum of patients with gastrointestinal tract tumors. Clinical Biochemistry, 41(10-11), 852-858.
21. Sharma, P., & Kumar, S. (2018). Metformin inhibits human breast cancer cell growth by promoting apoptosis via a ROS-independent pathway involving mitochondrial dysfunction: pivotal role of superoxide dismutase (SOD). Cellular Oncology, 41, 637-650.
22. Teodoro, A. J., Oliveira, F. L., Martins, N. B., Maia, G. D. A., Martucci, R. B., & Borojevic, R. (2012). Effect of lycopene on cell viability and cell cycle

23. progression in human cancer cell lines. Cancer cell international, 12, 1-9.
24. Ubezio, P., & Civoli, F. (1994). Flow cytometric detection of hydrogen peroxide production induced by doxorubicin in cancer cells. Free Radical Biology and Medicine, 16(4), 509-516.
25. Wang, L., Du, F., & Wang, X. (2008). TNF-α induces two distinct caspase-8 activation pathways. Cell, 133(4), 693-703.