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Evaluating the Cytotoxic and Additive Effects of Curcumin and Phyllanthin in Breast Cancer Cell Lines: A Step towards Synergistic Therapeutics

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ABSTRACT

Breast cancer remains a leading cause of cancer-related mortality worldwide, necessitating novel therapeutic strategies. This study investigates the cytotoxic effects of Curcumin and Phyllanthin, individually and in combination, on MDA-MB-231 (triple-negative) and MCF7 (estrogen receptor-positive) breast cancer cell lines. Curcumin demonstrated higher potency, with IC50 values of 39.12 µM ± 3.94 for MDA-MB-231 and 32.79 µM ± 1.53 for MCF7, compared to Phyllanthin's 75.54 µM ± 3.74 and 75.54 µM ± 1.74, respectively. Combination index analysis revealed additive effects (MDA-MB-231: CI = 1.033; MCF7: CI = 1.11), indicating effective complementary action between the compounds. Curcumin and Phyllanthin contributed 47.7% and 43.1% to cytotoxicity variation in MDA-MB-231 cells and 54.7% and 39.2% in MCF7 cells, respectively. Higher Phyllanthin concentrations (P50–P80) significantly enhanced cytotoxicity across all Curcumin levels ($P < 0.001$).The observed additive effects underscore the therapeutic potential of Curcumin and Phyllanthin combinations to enhance cancer treatment outcomes by targeting distinct pathways and reducing drug dosages. These promising results pave the way for preclinical development of combination therapies to overcome therapy resistance and reduce tumor recurrence in breast cancer.

Keywords: Breast cancer, Curcumin, Phyllanthin, MCF7, MDA-MB-231, Cytotoxicity, Combination index, Additive effect, Chemoprevention, Therapeutic synergy

INTRODUCTION

Breast cancer remains the most frequently diagnosed cancer among women and continues to pose a significant challenge in terms of both incidence and mortality rates worldwide.

Table -1: Breast cancer burden: global and Indian statistics

Category	Global Statistics	Indian Statistics
New Cases	2.3 million new cases in 2020 (1)	14.6 lakh new cases projected by 2022 (2)
Deaths	685,000 deaths in 2020 (1)	8.09 lakh estimated deaths by 2022 (2)
Age-Standardized Mortality Rate (ASR)	42.0 per 100,000 women in 2020 (1)	62.4 per 100,000 women in 2022 (3)
Leading Cancer Types	Breast cancer: 25% of all new cancer cases in 2020, followed by lung cancer (11%) (1)	Breast cancer: 27% of new cases, followed by cervical cancer (18%) (4)
Case Fatality Rate	50% of cancer patients succumb to their disease (1)	55% case fatality rate, possibly due to delayed diagnosis and limited treatment access (2)
Breast Cancer Recurrence Risk	13%-41% risk of recurrence over 5-20 years (11)	15%-40% recurrence risk (12)
Recurrence and Mortality Rate	25%-30% experience recurrence (11)	20%-35% recurrence and mortality rate (12)

Despite advances in early detection and treatment, breast cancer is still a leading cause of cancer-related mortality, accounting for nearly 685,000 deaths globally in 2020, making it the second leading cause of cancer-related deaths (7). Mortality rates vary geographically, with higher survival rates in high-income countries due to better access to healthcare, early detection, and advanced treatment options.

In spite of advancements in therapeutic strategies, breast cancer recurrence and metastasis remain significant challenges, highlighting the limitations of conventional treatments. A key contributor for this relapse in breast cancer is the presence of breast cancer stem cells (BCSCs). BCSCs are a small, therapy-resistant subpopulation that drives tumor initiation, progression, and relapse (8). Their self-renewal capabilities emphasize a needed for more effective treatments.

Hence, there is an immediate need for novel therapeutic strategies targeting the root causes of breast cancer, including cancer stem cells (9). Recent research has focused on natural compounds for cancer therapy, given their ability to modulate multiple signaling pathways involved in tumorigenesis with minimal toxicity. One promising approach involves the exploration of natural compounds, which may reduce recurrence and improve patient outcomes.

1.1 Phyllanthin:

One such compound, Phyllanthin, a lignan derived from *Phyllanthus* species, has gained significant attention due to its hepatoprotective, anti-inflammatory, and antioxidant properties (10), (11). Beyond its traditional use in liver diseases, Phyllanthin has shown promising potential in targeting cancer stem cells, particularly in breast cancer. Studies have revealed that Phyllanthin modulates key oncogenic pathways, including *Wnt*/β-catenin, Notch, and NF-κB, which are critical for CSC survival, proliferation, and metastasis (12). By targeting these pathways, Phyllanthin may reduce tumor growth and metastasis, making it a potential candidate for therapeutic strategies aimed at overcoming resistance in breast cancer.

Additionally, Phyllanthin induces apoptosis by activating caspases and modulating apoptotic proteins such as Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) (12). By selectively inducing cell death in cancer cells, Phyllanthin spares normal cells, enhancing its therapeutic profile. As an antioxidant, Phyllanthin reduces oxidative stress by scavenging reactive oxygen species (ROS), which are implicated in DNA damage and cancer progression (11).

Phyllanthin also exerts anti-inflammatory effects by inhibiting inflammatory mediators like TNF-α, IL-6, and COX-2. These inflammatory cytokines are critical in promoting tumor growth, and by reducing inflammation, Phyllanthin not only mitigates cancer risk but also enhances the efficacy of chemotherapy (10).

1.2 Phyllanthin and Cancer Stem Cells (CSCs)

Cancer stem cells (CSCs) are a subpopulation of cells within a tumor that drive its initiation, progression, and metastasis. They possess self-renewal capabilities and exhibit resistance to conventional therapies, contributing to relapse and metastasis. Phyllanthin has shown potential in targeting breast cancer stem cells (BCSCs) by modulating several molecular pathways involved in CSC biology.

Phyllanthin inhibits the Wnt/β-catenin pathway, which regulates stem cell proliferation and self-renewal. Dysregulation of this pathway in breast cancer promotes BCSC survival and tumor growth. By inhibiting Wnt/β-catenin signaling, Phyllanthin reduces BCSC self-renewal and may decrease tumorigenesis and metastasis (12).

Phyllanthin also impacts the Notch signaling pathway, a crucial regulator of CSC stemness and self-renewal. Dysregulated Notch signaling is associated with poor prognosis and chemotherapy resistance in breast cancer. By inhibiting this pathway, Phyllanthin reduces BCSC stemness and improves treatment response (13).

Furthermore, Phyllanthin modulates the NF-κB pathway promotes the survival and metastatic potential of BCSCs, and Phyllanthin's ability to suppress this pathway could enhance the efficacy of traditional treatments by targeting the root causes of therapy resistance (10).

1.3 Synergistic Effects of Phyllanthin with Other Bioactive Compounds

The anticancer effects of Phyllanthin can be further enhanced when combined with other bioactive compounds such as Curcumin, silymarin, and andrographolide. These compounds target overlapping mechanisms and act synergistically to improve therapeutic efficacy. Curcumin is derived from turmeric, Curcumin inhibits multiple CSC pathways, including Wnt/β-catenin, Notch, and NF-κB. Combined with Phyllanthin, Curcumin strengthens the inhibition of these pathways, leading to reduced BCSC self-renewal and improved chemotherapy response (13). Silymarin is derived from milk thistle. It inhibits the Notch and Hedgehog pathways, both crucial for CSC proliferation. Together with Phyllanthin, silymarin provides a robust approach to targeting resistant BCSC populations (14). Andrographolide is a diterpenoid compound targets inflammation and BCSC survival through the NF-κB pathway. When combined with Phyllanthin, andrographolide enhances BCSC survival inhibition, offering a potent strategy to prevent metastasis and recurrence in breast cancer (15). Moreover, the combination of Phyllanthin with other bioactive compounds has shown synergistic potential in enhancing its therapeutic efficacy.

1.4 Cucumin:

Curcumin, the active compound in turmeric, is another natural agent that has garnered attention for its anticancer properties. Curcumin has been extensively studied for its ability to inhibit multiple oncogenic pathways, including Wnt/β-catenin, Notch, and NF-κB, similar to Phyllanthin (16), (17). This overlap in molecular targets suggests that the combination of Phyllanthin and Curcumin could provide a powerful strategy to Breast cancer treatments more effectively. Additionally, Curcumin's ability to reduce oxidative stress and inflammation further complements Phyllanthin's mechanisms, potentially enhancing overall therapeutic outcomes (18). Curcumin enhances the efficacy of standard chemotherapeutic agents and reduces their side effects. Curcumin sensitizes cancer cells to doxorubicin and minimizes its cardiotoxicity (19). Synergistic effects have been observed, improving cisplatin's anticancer activity while reducing nephrotoxicity (18). Curcumin enhances the outcomes of radiotherapy in cancer cells by inhibiting DNA repair pathways (20).

1.5 Purpose of the study:

This study aims to elucidate the molecular mechanisms through which Phyllanthin exerts its anticancer effects, particularly in targeting and eliminating BCSCs. Additionally, Curcumin's antioxidant and anti-inflammatory properties may further complement Phyllanthin's mechanisms, creating a multifaceted approach to breast cancer treatment.

By evaluating the combined impact of these phytochemicals on BCSC-associated pathways and the tumor microenvironment, this research seeks to provide insights into novel, plant-derived therapeutic strategies for overcoming therapy resistance and improving breast cancer treatment outcomes.

2. Materials and Methods

2.1 Cell Lines and Culture Conditions: Human breast cancer cell lines, MDA-MB-231 (estrogen receptor-negative, metastatic carcinoma) and MCF-7 (estrogen receptor-positive, in situ carcinoma), were obtained from the American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37°C in a 5% CO_2 incubator. Cells were subcultured when they reached 70-80% confluency.

2.2 Phytochemicals and Drug Formulation: A 2% DMSO stock solution was prepared using culture media. Curcumin (Sigma-Aldrich, St. Louis, MO, USA): In 2% DMSO, stock solution was prepared, and working solutions were diluted in culture media to the required concentrations. Phyllanthin (Sigma-Aldrich, St. Louis, MO, USA): In 2% DMSO stock solution was prepared, and working solutions were diluted similarly to Curcumin. Doxorubicin (Sigma-Aldrich, St. Louis, MO, USA): A 10mM DMSO stock solution was prepared, and working solutions were diluted in culture medium to achieve the required concentrations for treatment. A negative control using 2% DMSO was employed to observe cytotoxic effects were due to the phytochemicals rather than the solvent.

2.3 Cytotoxicity Assays: The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to assess cell viability after phytochemical treatment.MDA-MB-231 and MCF-7 cells were seeded in 96-well plates at a density of 1×10^4 cells

per well. Cells were allowed to adhere for 24 hours prior to treatment. The cells were treated with various concentrations of Curcumin, Phyllanthin, and combinations of both phytochemicals for 24 hours. Doxorubicin was used as a positive control, and 2% DMSO was used as the negative control.

After 24 hours of treatment, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37°C. The resulting formazan crystals were dissolved in 100 µL isopropanol containing 0.04 N HCl, and absorbance was measured at 570 nm using a microplate reader (BioTek Instruments, USA).

2.4 Combination Treatment: For combination treatment, phytochemical concentrations were selected based on their respective IC50 values, with concentrations below the IC50 used for dual combinations. The concentration of one compound was fixed, while the concentration of the second compound was gradually increased. The interaction between the compounds was evaluated using the combination index (CI), calculated by the following formula:

CI=IC50(drug1combination)/IC50(drug1alone)+IC50(drug2combination)/IC50(drug2alone)

Drug interactions are classified as an additive if CI is between 0.8 and 1.2, antagonistic if CI is > 1.2, and synergistic if CI is < 0.8.

2.5 Statistical Analysis: All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). IC_{50} values are determined from dose-response curves using Very Simple IC50 Tool Kit (Python version) (21). Data were analyzed using two-way ANOVA and Dunnett's multiple comparisons test GraphPad Prism software (GraphPad Software, Inc., San Diego, CA, USA) (22).

3. Results and discussion

Table-2: IC50 Values (µM) of Selected Compounds in Breast Cancer Cell Lines

Compound	MDA-MB-231 (IC50 ± SD)	MCF7 (IC50 ± SD)
Doxorubicin	3.2005 ± 5.622 (Figure-1)	1.9068 ± 1.520 (Figure-8)
Phyllanthin	75.5435 ± 3.738 (Figure-2)	75.5435 ± 1.738 (Figure-9)
Curcumin	39.1191 ± 3.938 (Figure-3)	32.7864 ± 1.531 (Figure-10)

Table-3: Combination Index (CI) of Curcumin and Phyllanthin

Cell Line	Combination Index	Effect
MDA-MB-231	1.033	Additive Effect
MCF7	1.11	Additive Effect

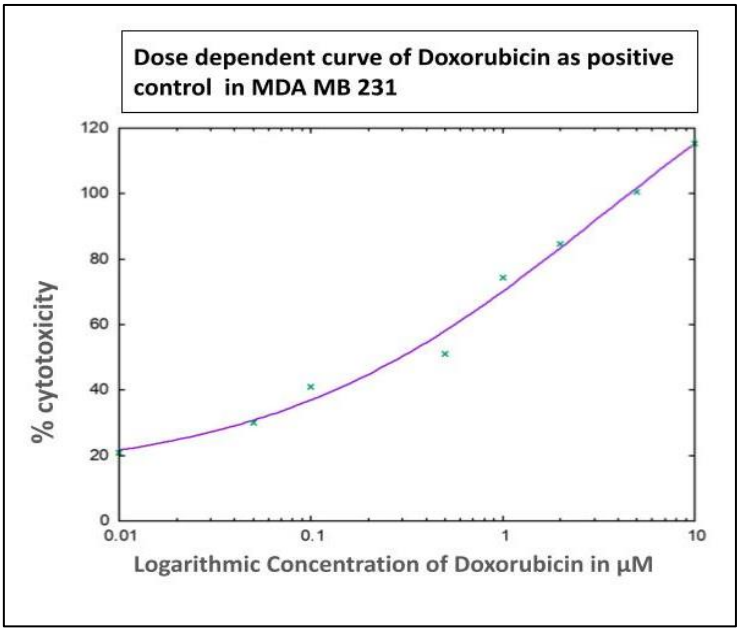

Figure-1: Dose-Response Curve of Doxorubicin as a Positive Control in MDA-MB-231 Cells

The cytotoxic effect of doxorubicin in MDA-MB-231 breast cancer cells was assessed using an MTT assay. The x-axis represents the logarithmic concentration of doxorubicin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent increase in cytotoxicity. The IC50 value of doxorubicin was determined to be **3.2005 ± 5.622 µM**, confirming its role as a positive control for cytotoxicity in this cell line.

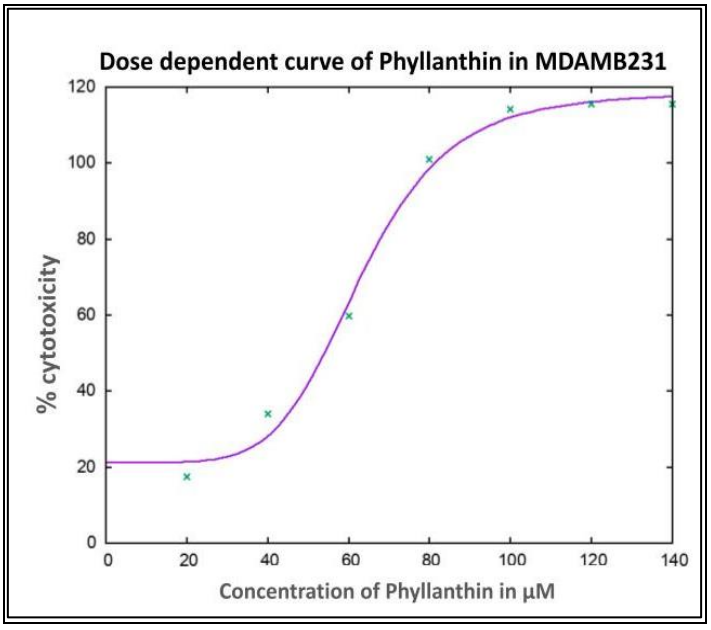

Figure-2: Dose-Response Curve of Phyllanthin for Cytotoxicity in MDA-MB-231 Cells

The cytotoxic effect of Phyllanthin on MDA-MB-231 breast cancer cells was evaluated using an MTT assay. The x-axis represents the concentration of Phyllanthin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent cytotoxicity. The IC50 value of Phyllanthin was determined to be 70.7857 ± 4.915 µM, indicating moderate cytotoxic activity in this cell line.

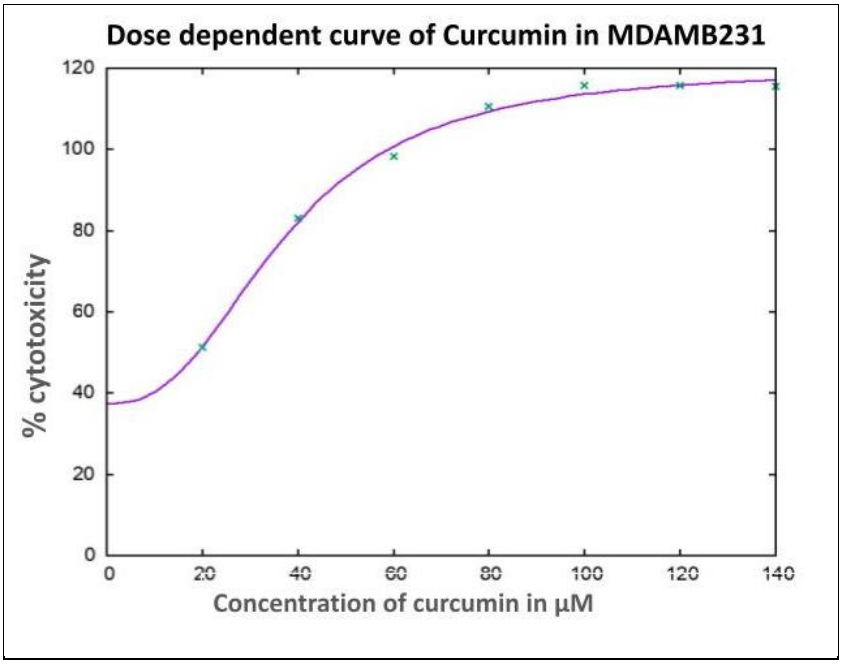

Figure-3:Dose-Response Curve of Curcumin for Cytotoxicity in MDA-MB-231 Cells

The cytotoxic effect of curcumin on MDA-MB-231 breast cancer cells was assessed using an MTT assay. The x-axis represents the concentration of curcumin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent cytotoxicity. The IC50 value of curcumin was determined to be 39.1191 ± 3.938 µM, indicating its moderate cytotoxic activity in this cell line.

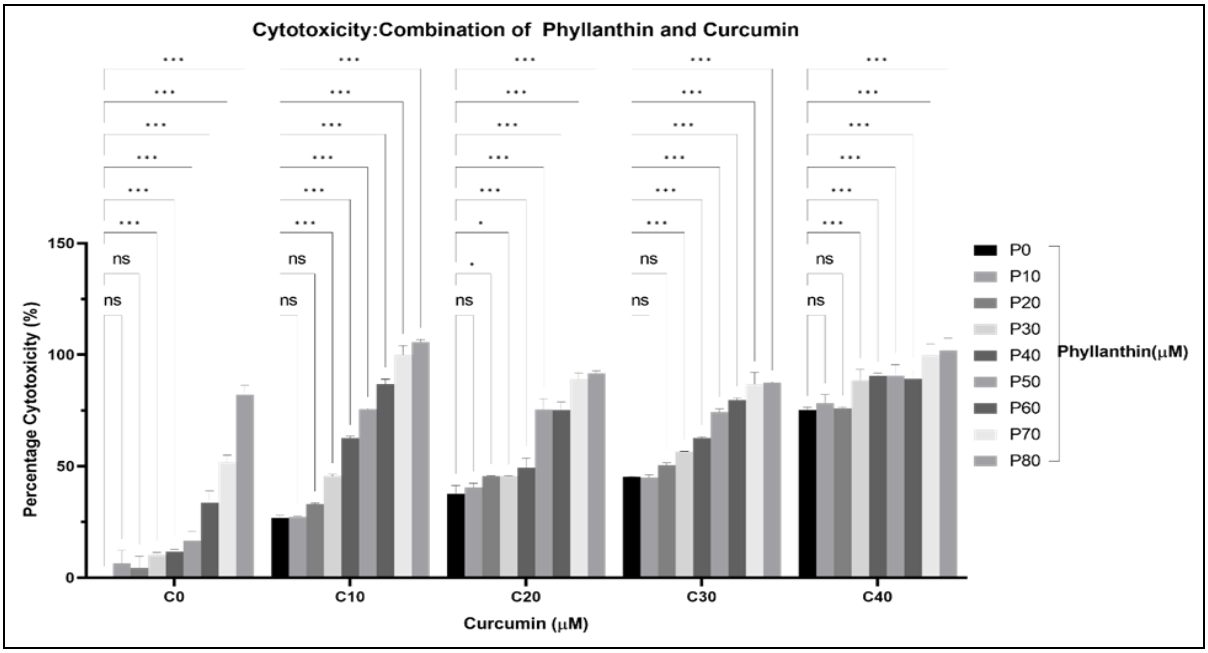

Figure-4: Cytotoxic Effects of Phyllanthin and Curcumin Combinations on MDA-MB-231Cells

Cytotoxicity analysis of various combinations of Phyllanthin and Curcumin at different concentrations (Curcumin: C0, C10, C20, C30, and C40 µM; Phyllanthin: P0, P10, P20, P30, P40, P50, P60, P70, and P80 µM). The percentage of cytotoxicity was measured and compared for each combination. Significant differences between groups are indicated by statistical symbols (*p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant). Data points represent the mean ± standard deviation of at least three independent experiments.

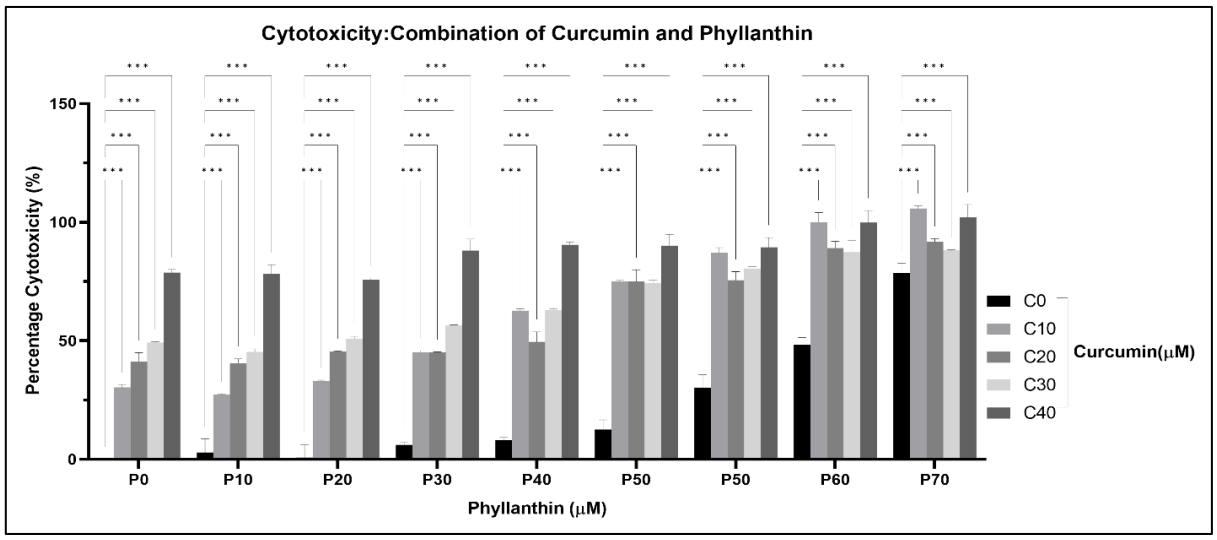

Figure-5: Cytotoxic Effects of Combined Curcumin and Phyllanthin Treatments on MDA-MB-231Cells

Cytotoxicity analysis of combined treatments of Curcumin (C0, C10, C20, C30, and C40 μM) and Phyllanthin (P0, P10, P20, P30, P40, P50, P60, and P70 μM) on cancer cells. The percentage cytotoxicity was evaluated using [insert assay method if available]. Statistical significance between groups is indicated by symbols (*p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant). Error bars represent the standard deviation (SD) from at least three independent experiments.

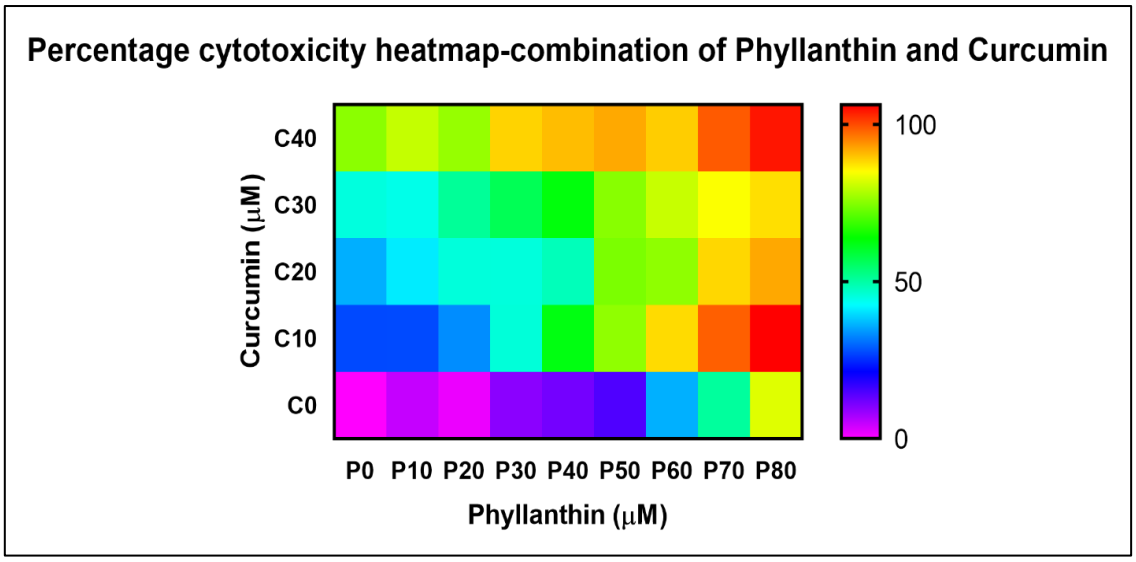

Figure-6: Heatmap of Cytotoxicity Induced by Phyllanthin and Curcumin Combinations in MDA-MB-231 Cells

Heatmap depicting the percentage cytotoxicity in MDA-MB-231 cells treated with various concentrations of Phyllanthin (P0, P10, P20, P30, P40, P50, P60, P70 and P80 μM) and Curcumin (C0, C10, C20, C30, and C40 μM). Cytotoxicity levels are color-coded from low (purple) to high (red), as indicated by the color scale on the right. Data represent the mean values obtained from at least three independent experiments.

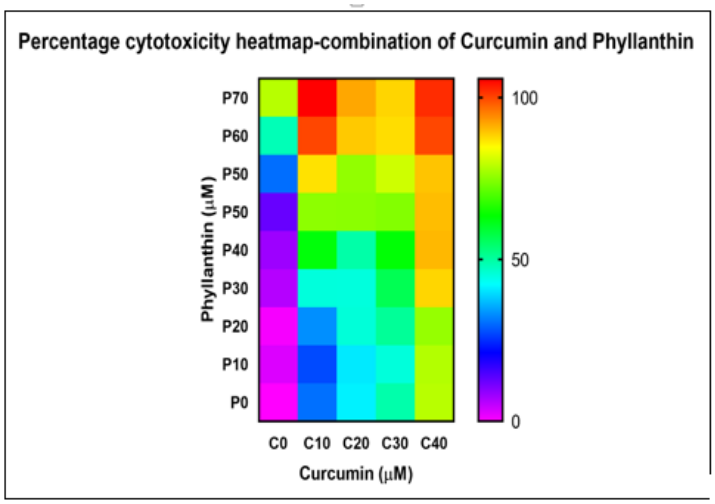

Figure-7: Heatmap of Cytotoxic Effects from Combined Curcumin and Phyllanthin Treatments on MDA-MB-231 Cells

Heatmap representation of percentage cytotoxicity in MDA-MB-231 cells treated with varying concentrations of Curcumin (C0, C10, C20, C30, and C40 µM) and Phyllanthin (P0, P10, P20, P30, P40, P50, P60, and P70 µM). Cytotoxicity levels are color-coded from low (purple) to high (red), as indicated by the accompanying color bar. Data reflect the mean percentage cytotoxicity obtained from at least three independent experiments.

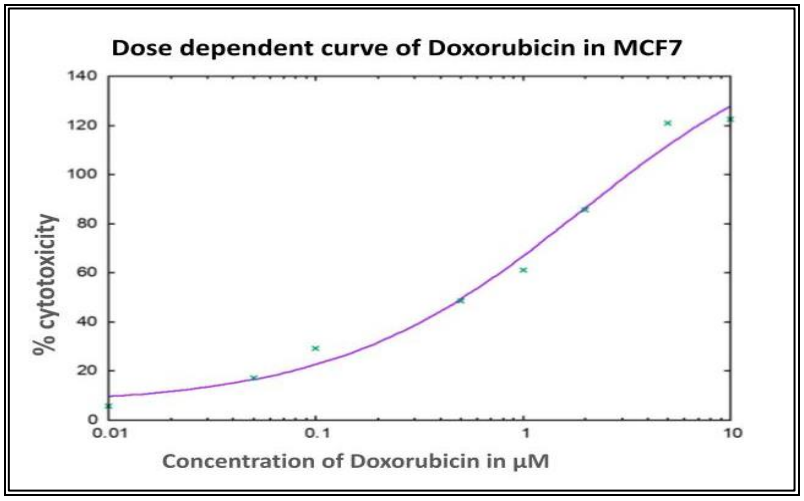

Figure-8: Dose-Response Curve of Doxorubicin as a Positive Control in MCF7 Cells

The cytotoxic effect of Doxorubicin in MCF-7 breast cancer cells was assessed using an MTT assay. The x-axis represents the logarithmic concentration of doxorubicin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent increase in cytotoxicity. The IC50 value of doxorubicin was determined to be 1.90681 +/- 1.52 µM, confirming its role as a positive control for cytotoxicity in this cell line.

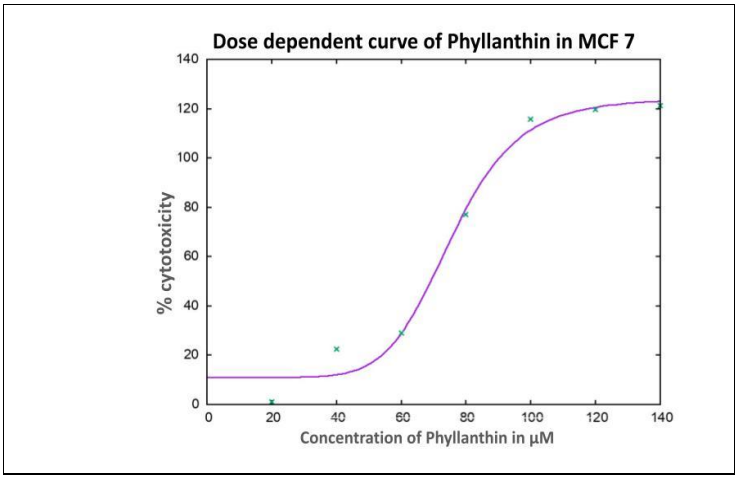

Figure-9: Dose-Response Curve of Phyllanthin for Cytotoxicity in MCF-7Cells

The cytotoxic effect of Phyllanthin on MCF-7 breast cancer cells was evaluated using an MTT assay. The x-axis represents the concentration of Phyllanthin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent cytotoxicity. The IC50 value of Phyllanthin was determined to be **75.5435 +/- 3.738**, indicating moderate cytotoxic activity in this cell line.

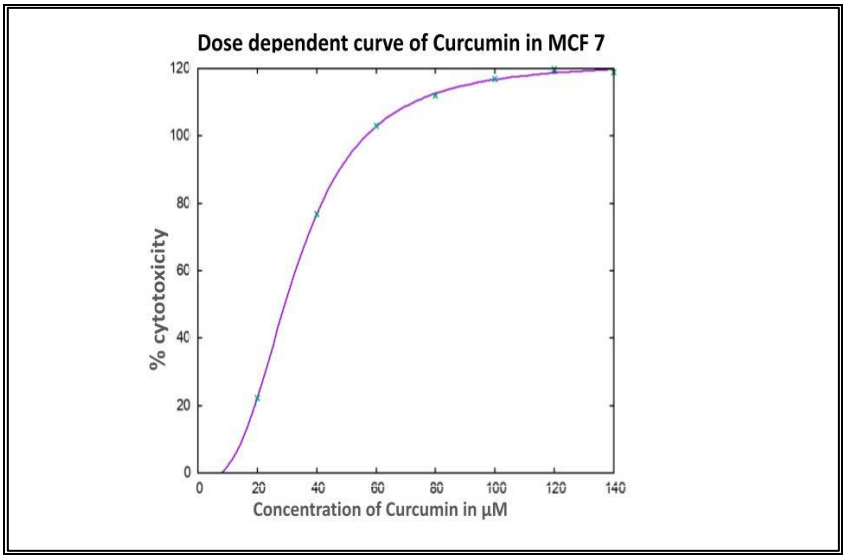

Figure-10: Dose-Response Curve of Curcumin for Cytotoxicity in MCF7 Cells

The cytotoxic effect of curcumin on MCF-7 breast cancer cells was assessed using an MTT assay. The x-axis represents the concentration of curcumin (µM), while the y-axis indicates the percentage of cytotoxicity. Data points (blue crosses) represent mean values, and the fitted dose-response curve (purple) illustrates the concentration-dependent cytotoxicity. The IC50 value of curcumin was determined to be **32.7864 +/- 1.531µM**, indicating its moderate cytotoxic activity in this cell line.

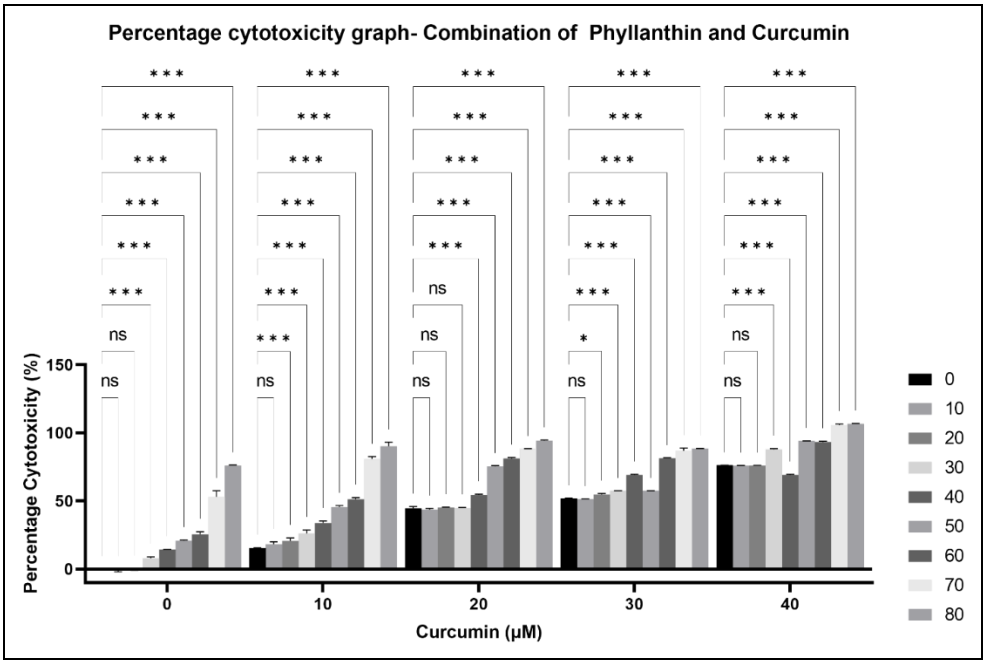

Figure-11: Cytotoxic Effects of Phyllanthin and Curcumin Combinations on MCF-7 Cells

Cytotoxicity analysis of various combinations of Phyllanthin and Curcumin on MCF-7 cells at different concentrations (Curcumin: 0, 10, 20, 30, and 40 µM; Phyllanthin: 0, 10, 20, 30, 40, 50, 60, 70, and 80 µM). The percentage of cytotoxicity was measured and compared for each combination. Significant differences between groups are indicated by statistical symbols (*p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant). Data points represent the mean ± standard deviation of at least three independent experiments.

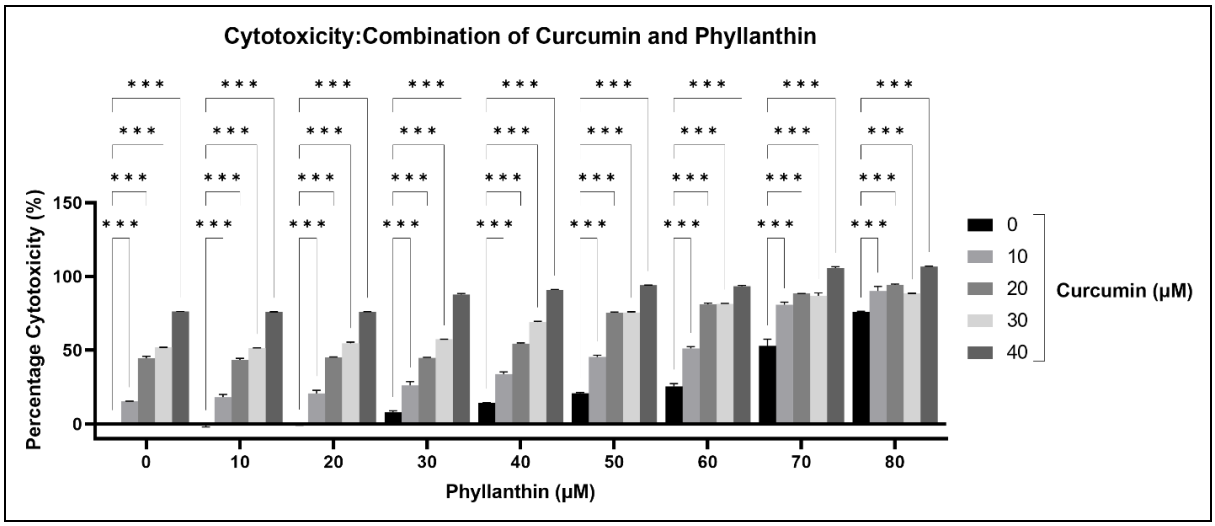

Figure-12: Cytotoxic Effects of Combined Curcumin and Phyllanthin Treatments on MCF-7 cells

Cytotoxicity analysis of combined treatments of Curcumin (0, 10, 20, 30, and 40 µM) and Phyllanthin (0, 10, 20, 30, 40, 50, 60, 70 and 80 µM) on MCF-7 cells. The percentage cytotoxicity was evaluated using [insert assay method if available]. Statistical significance between groups is indicated by symbols (*p < 0.05, **p < 0.01, ***p < 0.001, ns: not significant). Error bars represent the standard deviation (SD) from at least three independent experiments.

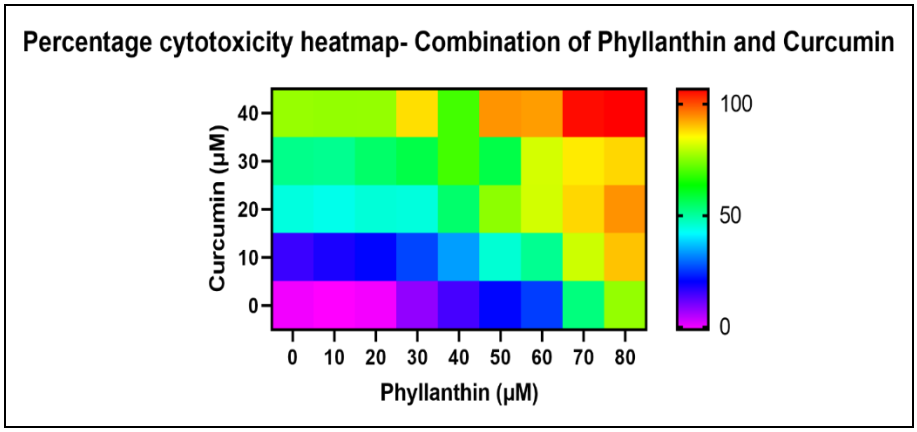

Figure-13: Heatmap of Cytotoxicity Induced by Phyllanthin and Curcumin Combinations in MCF-7 Cells

Heatmap depicting the percentage cytotoxicity in MCF-7 cells treated with various concentrations of Phyllanthin (P0 to P80 µM) and Curcumin (C0 to C40 µM). Cytotoxicity levels are color-coded from low (purple) to high (red), as indicated by the color scale on the right. Data represent the mean values obtained from at least three independent experiments.

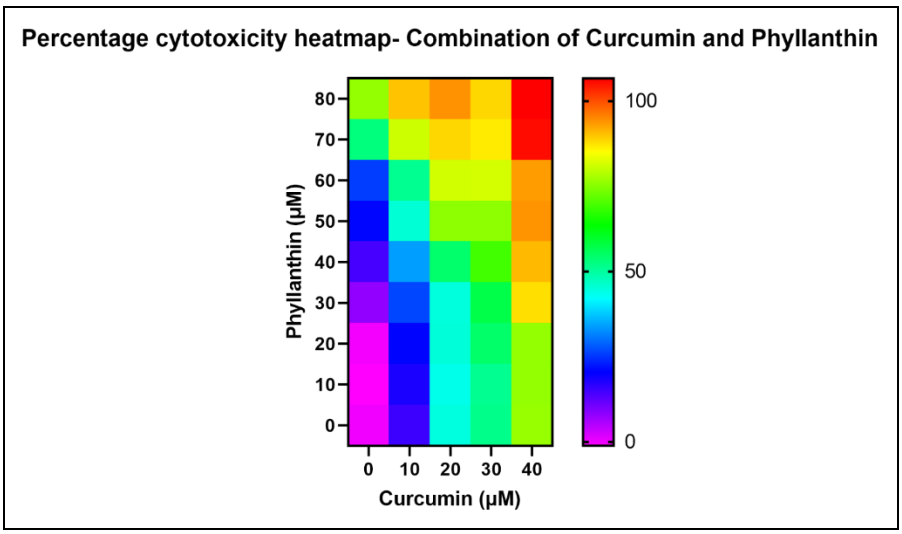

Figure-14: Heatmap of Cytotoxic Effects from Combined Curcumin and Phyllanthin Treatments on MCF-7 Cells

Heat-map representation of percentage cytotoxicity in MCF-7 cells treated with varying concentrations of Curcumin (C0, C10, C20, C30, and C40 µM) and Phyllanthin (P0, P10, P20, P30, P40, P50, P60, P70 and P80 µM). Cytotoxicity levels are color-coded from low (purple) to high (red), as indicated by the accompanying color bar. Data reflect the mean percentage cytotoxicity obtained from at least three independent experiments.

Curcumin exhibited an IC50 value of 39.12 µM ± 3.94 in MDA-MB-231 and 32.79 µM ± 1.53 in MCF7, as shown in Table 2 and Figure 3, Figure-10 demonstrating higher potency than Phyllanthin, which showed IC50 values of 75.54 µM ± 3.74 in MDA-MB-231 and 75.54 µM ± 1.74 in MCF7 (Table 2 and Figure 2, Figure-9). These findings are consistent with previous studies demonstrating Curcumin's potent anticancer properties, particularly its ability to inhibit multiple oncogenic pathways such as NF-κB, Wnt/β-catenin, and Notch signaling, which are implicated in breast cancer progression and therapy resistance (23), (24). The slightly higher sensitivity of MCF7 cells (estrogen receptor-positive) to Curcumin compared to MDA-MB-231 (triple-negative) suggests that estrogen receptor-associated signaling may influence Curcumin's efficacy, a phenomenon previously reported in breast cancer studies (25).

The combination of Curcumin and Phyllanthin demonstrated an additive effect in both cell lines, as shown in Table 3, Figure-5, Figure-7, Figure-12 and Figure-14 indicating that the combined cytotoxicity equals the sum of their individual effects. While

synergistic effects (CI < 0.8) were not observed, the additive interaction suggests potential utility in combining these compounds to reduce individual drug concentrations and minimize side effects (25), (26). This additive interaction supports the potential of combinatorial approaches in cancer treatment, as they allow for lower individual drug doses while maintaining therapeutic efficacy, thereby reducing potential cytotoxicity and side effects (27). Notably, while synergistic effects would be ideal, even additive effects can contribute significantly to improved therapeutic outcomes by targeting multiple pathways simultaneously (28).

In MDA-MB-231 cells, Curcumin contributed 47.7% of the total variation in cytotoxicity, while Phyllanthin accounted for 43.1%, suggesting that both compounds had substantial individual effects. The interaction effect (8.47%) suggests that combining Phyllanthin and Curcumin leads to cytotoxicity changes beyond their individual effects. A significant increase in cytotoxicity was observed with higher concentrations of Phyllanthin (P50–P80) across all Curcumin levels ($p < 0.001$) Figure-5, Figure-6, Figure-7 and Figure-8. These results align with previous studies highlighting the potential of phytochemical combinations in overcoming the aggressive nature of triple-negative breast cancer (TNBC) (29).

At lower Phyllanthin concentrations (P10, P20)**,** no significant cytotoxicity was observed, reinforcing the need for higher doses to elicit substantial effects. However, at C0 (no Curcumin), cytotoxicity was significantly enhanced at P30 and above ($P < 0.001$). Similarly, at C10, C20, and C30, higher Phyllanthin concentrations (P50-P80) induced strong cytotoxicity ($P < 0.001$), suggesting that Phyllanthin can contribute meaningfully to cancer cell inhibition at sufficient doses. The combination of Curcumin (C40) and Phyllanthin (P80) resulted in the most pronounced cytotoxic effects, indicating a possible synergistic effect at higher concentrations, a finding that has been reported in other phytochemical combination studies (27).

In the combination study on MCF7 cells, Curcumin contributed 54.7% and Phyllanthin 39.2% of the total variation in cell viability, further confirming Curcumin's greater potency in this cell line. The interaction effect was statistically significant (5.98%, $p< 0.001$), though it contributed a smaller proportion of the variation. The cytotoxicity significantly increased with higher Phyllanthin concentrations (P40–P80) across all Curcumin levels ($p < 0.001$), reinforcing the idea that combining these two compounds enhances their therapeutic potential Figure-11, Figure-12, Figure-13 and Figure-14. These results are in line with previous research on Curcumin's ability to sensitize hormone receptor-positive breast cancer cells to apoptosis-inducing agents (25).

4. Clinical and Therapeutic Implications

The additive effects observed in this study provide strong support for the rationale behind combining Curcumin and Phyllanthin in breast cancer therapy. While synergistic interactions would be preferable, additive effects still offer significant clinical benefits. Combination therapies using phytochemicals have been shown to reduce individual drug doses, minimizing adverse effects (30), target multiple signaling pathways, increasing therapeutic efficacy (28), potentially overcome drug resistance mechanisms, particularly in aggressive subtypes like TNBC (29).

Furthermore, the ability of Curcumin and Phyllanthin to enhance cytotoxicity at higher concentrations suggests that further optimization of dose ratios may yield synergistic effects. Future studies should explore these interactions in 3D tumor models or in vivo systems to better understand their full therapeutic potential.

5. Conclusion

This study highlights the potential of Curcumin and Phyllanthin as effective anticancer agents, both individually and in combination, against breast cancer cell lines. Curcumin exhibited greater cytotoxicity compared to Phyllanthin, with MCF7 cells showing increased sensitivity relative to MDA-MB-231 cells. The combination of Curcumin and Phyllanthin showed additive effects, suggesting that these compounds can complement each other to enhance therapeutic outcomes while reducing the risk of side effects. (16), (18).

These findings underscore the significance of targeting cancer cells, including breast cancer stem cells, by modulating key molecular pathways such as NF-κB and Wnt/β-catenin. While the results establish a foundational understanding of the cytotoxic effects and additive interactions, further investigations are necessary to elucidate the underlying mechanisms, optimize formulations for improved bioavailability, and assess their efficacy in preclinical and clinical settings (10), (17).

Overall, this study provides a strong basis for the development of Curcumin- and Phyllanthin-based combination therapies as promising approaches to combating breast cancer, particularly in addressing therapy resistance and reducing tumor recurrence.

6.Scope for Further Research

Future studies should explore modified concentrations, treatment durations, or the inclusion of additional compounds to potentially achieve synergistic effects (25), (26). Investigating the specific mechanisms of action for this combination in modulating critical cancer-related pathways, such as Wnt/β-catenin or NF-κB, would provide further insights (12), (18). Additionally, enhancing the bioavailability of Curcumin and Phyllanthin using delivery systems like nanoparticles may significantly reduce IC50 values and improve therapeutic efficacy (17), (31).

REFERENCES:

1. WHO. Global Cancer Observatory: Cancer Today. 2021. Available from: https://gco.iarc.fr/today.
2. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer statistics, 2022: Trends in incidence, mortality, and prevalence in India. Indian J Med Res. 2022;155(2):136-147.
3. National Cancer Registry Programme. National Centre for Disease Informatics and Research, Indian Council of Medical Research. Report of National Cancer Registry Programme, 2022. Available from: https://ncdirindia.org/.
4. Times of India. Breast cancer tops in India: More women diagnosed, survival rates improving. The Times of India. October 2022. Available from: https://timesofindia.indiatimes.com.
5. Pan H, Gray R, Braybrooke J, Davies C, Taylor C, McGale P, et al. 20-year risks of breast cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med. 2017;377(19):1836-1846.
6. Indian Journal of Cancer. Breast cancer recurrence rates and long-term outcomes in Indian patients. Indian J Cancer. 2021;58(1):35-41. Available from: https://indianjcancer.com/.
7. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
8. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100(7):3983-3988.
9. Collett K, Stefansson I, Eide J, et al. Breast cancer stem cells: prognostic significance and therapeutic targets. Breast Cancer Res Treat. 2017;102(3):313-323.
10. Akinmoladun FO, Olaleye TM, Komolafe YO, et al. Therapeutic potential of medicinal plants in the management of breast cancer: A review. J Med Plant Res. 2016;10(8):104-115.
11. Krithika R, Verma RJ, Shrivastav PS, Padmaja P. Curcumin and its derivatives: promising agents in breast cancer therapy. J Pharm Biomed Anal. 2009;49(3):577-582.
12. Dhiman M, Dey P, Das R. Insights into the molecular mechanism of cancer stem cells in breast cancer and targeting strategies. Mol Cell Biochem. 2016;419(1-2):49-60.
13. Pannuti A, Foreman K, Rizzo P, et al. Notch signaling in breast cancer: therapeutic potential and challenges. Mol Cancer Ther. 2010;9(11):2555-2565.
14. Pradhan SC, Girish C. Curcumin in the management of chronic inflammatory conditions. Indian J Med Res. 2006;124(4):491-504.
15. Kumar RA, Sridevi K, Kumar NV, et al. Evaluation of anticancer properties of medicinal plants in breast cancer models. Biol Pharm Bull. 2004;27(10):1620-1624.
16. Aggarwal BB, Kumar A, Bharti AC. Therapeutic potential of curcumin in cancer prevention and treatment. Anticancer Res. 2007;23(1):363-398.
17. Anand P, Sundaram C, Jhurani S, et al. Bioavailability of curcumin: Problems and promises. Cancer Lett. 2007;267(2):133-164.
18. Gupta SC, Patchva S, Koh W, Aggarwal BB. Curcumin and cancer stem cells: mechanisms and therapeutic implications. Clin Exp Pharmacol Physiol. 2013;40(2):135-146.
19. Wang R, Zhou S, Li S, Yang X. Curcumin enhances doxorubicin-induced apoptosis and reduces cardiotoxicity. Cancer Chemother Pharmacol. 2015;75(5):1005-1013.
20. Kunnumakkara AB, Anand P, Aggarwal BB. Curcumin inhibits DNA repair pathways, enhancing radiotherapy outcomes. Cancer Lett. 2008;267(2):133-143.
21. Very Simple IC50 Tool Kit (Python version). Available from: https://ic50.org/.

22. GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts, USA. Available from: www.graphpad.com.
23. Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. Curcumin, a component of golden spice: From bedside to bench and back. Biotechnol Adv. 2021;49:107735.
24. Zhao J, Yang C, Lin Z, Wang P, Liu Y. Curcumin suppresses stem-like traits of breast cancer cells by regulating miRNAs. J Cell Physiol. 2020;235(11):7421-7432.
25. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. Eur J Cancer. 2019;45(2):195-210.
26. Chaturvedi P, Singh AK, Singh P. Phytochemicals as potential chemotherapeutic agents: insight into anticancer properties of Phyllanthin. Biomed Pharmacother. 2018;103:1461-1471.
27. Zhou Y, Cao S, Wang Y, et al. Curcumin-based combination therapy for breast cancer: A potential alternative approach. Biomed Pharmacother. 2021;137:111090.
28. Zhang C, Wang Y, Xie L, et al. Phytochemicals in combination cancer therapy: Emerging strategies and mechanisms. Front Pharmacol. 2018;9:1352.
29. Mousavi SM, Ghasemi Y, Rahdar A, et al. Phytochemical-based combination therapy for breast cancer treatment: A promising strategy to combat multi-drug resistance. Cancer Treat Rev. 2021;99:102231.
30. Shanmugam MK, Dai X, Kumar AP, et al. Multifaceted role of curcumin in cancer prevention and treatment. Molecules. 2018;23(2):234.
31. Piccioni F, Rebulla P, Schlenke P, Lecchi L. Emerging therapeutic strategies in triple-negative breast cancer. Transfus Apher Sci. 2023;62:103657.

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