GMR
The Original

DEVELOPMENT OF QUERCETIN-RESVERATROL IMMEDIATE RELEASE ANTICANCER TABLETS FOR THE MANAGEMENT OF BREAST CANCER BY INVESTIGATING CYTOTOXIC POTENTIAL AGAINST MCF-7 CELL LINES

Mohini Kuchekar[1], Omprakash Swami[2], Tushar Dongare[2], Priti Nigade[2], Sakshi Thete[2], Upendra Galgatte[3], Praveen Chaudhari[3], Bhushan Pimple[1]*

[1] Department of Pharmacognosy, PES Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India 411044
[2] Department of Quality Assurance, PES Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India 411044
[3] Department of Pharmaceutics, PES Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India 411044
* Corresponding Author: Dr. Bhushan Prakash Pimple, Email: pimplebhushan@yahoo.co.in

ABSTRACT
Breast cancer remains one of the most prevalent malignancies and a leading cause of cancer-related mortality among women worldwide, highlighting the need for effective and safer therapeutic approaches. The present study aimed to develop and optimize a quercetin–resveratrol immediate-release tablet for potential breast cancer management by integrating molecular docking, in vitro biological evaluation, pharmaceutical formulation, and Quality by Design (QbD)-based optimization. Molecular docking was initially performed to evaluate the interactions of selected phytoconstituents with the epidermal growth factor receptor (EGFR), identifying quercetin, resveratrol, and naringenin as promising candidates for further investigation. The antiproliferative activity of these phytoconstituents was subsequently assessed against MCF-7 breast cancer cells using the MTT assay, where quercetin and resveratrol exhibited greater cytotoxic activity than naringenin and were therefore selected for formulation development. Immediate-release tablets containing quercetin and resveratrol in different ratios (1:1, 1:2, and 2:1) were prepared using a solid dispersion approach followed by direct compression. Formulation optimization was carried out using a Box–Behnken Design to evaluate the influence of formulation variables on drug release, disintegration time, and tablet hardness. The optimized formulation demonstrated satisfactory physicochemical properties, acceptable pre- and post-compression characteristics, drug–excipient compatibility, and efficient dissolution. Biological evaluation of the developed formulations using the sulforhodamine B (SRB) assay demonstrated that the quercetin: resveratrol (2:1) formulation exhibited the greatest antiproliferative activity against MCF-7 cells, with an IC_{50} value comparable to that of gefitinib. Overall, the findings demonstrate that integrating computational screening, experimental validation, pharmaceutical formulation, and QbD-guided optimization provide a systematic strategy for developing phytoconstituent-based oral formulations and identifies the optimized quercetin–resveratrol immediate-release tablet as a promising candidate for further preclinical evaluation in breast cancer therapy.

KEYWORDS: Breast cancer; MCF-7 cellline, Quercetin; Resveratrol; EGFR; Molecular docking; Quality by Design (QbD); Immediate-release tablet.

1. INTRODUCTION

Breast cancer remains the most diagnosed malignancy and one of the leading causes of cancer-related deaths among women worldwide, posing a major public health challenge despite significant advances in early detection and treatment [1–4]. In addition, breast cancer progression is driven by multiple dysregulated signalling pathways, particularly epidermal growth factor receptor (EGFR) and phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), which regulate tumour cell proliferation, survival, invasion, metastasis, and therapeutic resistance [5–10]. These limitations have increased interest in safer therapeutic agents capable of simultaneously modulating multiple molecular pathways [5–10]. Naturally occurring phytochemicals have gained considerable attention because of their diverse pharmacological activities and favourable safety profiles [11–22]. Flavonoids, polyphenols, alkaloids, and other plant-derived compounds have demonstrated antiproliferative, pro-apoptotic, antioxidant, anti-inflammatory, and anti-metastatic activities through the regulation of multiple signalling pathways involved in carcinogenesis [11–22]. Comparative analysis of binding affinity and protein–ligand interactions enabled the selection of quercetin, resveratrol, and naringenin for further investigation. To facilitate oral delivery, immediate-release tablets were selected because of their rapid drug release, dose accuracy, ease of administration, patient convenience, and simple manufacturing process [27–29]. Individual phytoconstituents were subsequently evaluated against the MCF-7 human

breast adenocarcinoma cell line using the MTT assay to identify the compounds with the greatest antiproliferative activity [23,31,32]. Based on these findings, quercetin and resveratrol were selected for combination formulation studies, whereas naringenin was excluded because of its comparatively lower cytotoxic activity. Owing to the poor aqueous solubility and limited oral bioavailability of quercetin and resveratrol, solid dispersion technology followed by direct compression was employed for the development of immediate-release tablet formulations [14,15,27–30]. Prototype combination formulations containing quercetin and resveratrol in the ratios of 1:1, 1:2, and 2:1 were subsequently developed and optimized using the Quality by Design (QbD) approach based on a Box–Behnken Design (BBD) to evaluate the influence of critical formulation variables on key pharmaceutical quality attributes, including drug release, tablet hardness, and disintegration time [24–30]. Following pharmaceutical evaluation, the optimized combination formulations were assessed for their antiproliferative activity against MCF-7 cells using the sulforhodamine B (SRB) assay to identify the formulation exhibiting the greatest biological activity [33]. The optimized Quercetin: Resveratrol (2:1) immediate-release tablet formulation demonstrated superior cytotoxic activity and was selected as the lead formulation for further investigation.

The present study aimed to identify promising phytochemical candidates through molecular docking, evaluate their individual cytotoxic activity, develop and optimize quercetin–resveratrol immediate-release tablet formulations using a systematic Quality by Design approach, and subsequently assess the biological performance of the optimized formulations against MCF-7 breast cancer cells as a potential oral therapeutic strategy for breast cancer management.

2. MATERIALS AND METHODS

Chemicals and Reagents

Quercetin, resveratrol, and naringenin were obtained from Yucca Enterprises (Mumbai, India) and served as the phytochemical candidates investigated throughout the study. Gefitinib and everolimus were employed as the reference compounds during molecular docking against the epidermal growth factor receptor (EGFR) and phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt), respectively [5–10]. Gefitinib was also used as the reference drug during the MTT and sulforhodamine B (SRB) cytotoxicity assays. The human breast adenocarcinoma cell line (MCF-7) was procured from the National Centre for Cell Science (NCCS), Pune, India. Cell culture was carried out using Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic–antimycotic solution. Phosphate-buffered saline (PBS), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sulforhodamine B (SRB), acetic acid, and Tris-base were used during the cytotoxicity studies. Microcrystalline cellulose (MCC), sodium starch glycolate (SSG), polyvinylpyrrolidone K30 (PVP K30), lactose monohydrate, mannitol, talc, and magnesium stearate were used for the preparation of immediate-release tablet formulations. Methanol and all other chemicals and solvents used during the investigation were of analytical reagent grade. Molecular docking was performed using AutoDock Vina (version 1.2.0), and protein–ligand interactions were analysed using BIOVIA Discovery Studio Visualizer 2021. UV–Visible spectroscopic analysis was carried out using a Shimadzu UV-1800 spectrophotometer, while Fourier transform infrared (FTIR) spectra were recorded using a PerkinElmer Spectrum Two FTIR spectrophotometer. Tablet compression was performed using a rotary tablet compression machine, and dissolution studies were conducted using a USP Type II dissolution apparatus. Cell culture experiments were carried out in a Class II biosafety cabinet using a carbon dioxide (CO_2) incubator (Thermo Fisher Scientific), and absorbance for the MTT and SRB assays was measured using an ELISA microplate reader (Bioline BD 206). Formulation optimization and statistical analysis were performed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA).

Molecular Docking Studies

A literature-guided strategy was adopted to evaluate the interaction of the selected phytochemicals with two therapeutically important targets involved in tumour growth, progression, metastasis, and therapeutic resistance [5–10].

Selection of Molecular Targets

EGFR and PI3K/Akt were selected because of their established involvement in breast cancer cell proliferation, survival, invasion, metastasis, and therapeutic resistance [5–10]. The crystal structures of EGFR (PDB ID: 1M17) and PI3K/Akt (PDB ID: 3M77) were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). Gefitinib and everolimus were included as reference compounds for comparative docking against EGFR and PI3K/Akt, respectively [5–10].

Molecular Docking Procedure

Docking simulations were carried out using AutoDock Vina to evaluate the interaction of the selected phytochemicals with EGFR and PI3K/Akt. The docking grid was generated around the active binding region defined by the co-crystallized ligand of each receptor. Each prepared ligand was docked against the corresponding protein, and the generated binding poses were ranked according to their predicted binding free energy. The complexes exhibiting the

most favourable binding energies were selected for further interpretation. Protein–ligand interactions, including hydrogen bonding, hydrophobic interactions, π–π interactions, and π–alkyl interactions, were visualized using BIOVIA Discovery Studio Visualizer [5–10].

MTT Cytotoxicity Assay

The antiproliferative activity of the selected phytochemicals, namely quercetin, resveratrol, and naringenin, was evaluated against MCF-7 cells using the MTT assay. Gefitinib was included as the reference drug. The assay was carried out according to a reported method with minor modifications [31,32].

Development of Combination Immediate-Release Tablet Formulations

Based on the MTT cytotoxicity findings, quercetin and resveratrol were selected for the development of combination immediate-release tablet formulations. To investigate the influence of different drug combinations, three formulations containing quercetin and resveratrol in the ratios of 1:1, 1:2, and 2:1 were designed. The composition of the formulations is presented in Table 1. These formulations were prepared for subsequent Quality by Design (QbD)-based optimization and pharmaceutical evaluation before biological assessment [27–30].

Table 1. Composition of Quercetin–Resveratrol Combination Immediate-Release Tablet Formulations

Excipient	Functional role	Purpose in formulation
Quercetin Resveratrol	Active Pharmaceutical ingredient (API)	Anticancer activity
Microcrystalline cellulose (MCC)	Binder	Improves compressibility and tablet strength
Sodium Starch glycolate	Super-disintegrant	Facilitates rapid tablet disintegration
PVPK30	Solubility enhancer	Improves cohesion and mechanical strength
Mannitol	Diluent/ filler	Increases tablet bulk and improves mouth feel
Talc	Glident	Enhances powder flow
Magnesium stearate	Lubricant	Reduces friction during compression

Quality by Design (QbD)-Based Formulation Optimization

The combination immediate-release tablet formulations containing quercetin and resveratrol in the ratios of 1:1, 1:2, and 2:1 were optimized using the Quality by Design (QbD) approach to systematically evaluate the influence of critical formulation variables on the pharmaceutical quality of the developed formulations [24–30]. A three-factor, three-level Box–Behnken Design (BBD) was employed to investigate the effects of sodium starch glycolate (SSG), microcrystalline cellulose (MCC), and polyvinylpyrrolidone K30 (PVP K30) on the critical quality attributes of the immediate-release tablets (Table 2). The experimental design consisted of seventeen runs generated using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). The selected responses included percentage drug release of quercetin, percentage drug release of resveratrol, tablet hardness, and disintegration time. The experimental data were evaluated using polynomial regression analysis, response surface methodology, and numerical optimization to establish the relationship between the formulation variables and the selected responses and to identify the optimized formulation [24–30].

Table 2. Independent Variables and Responses Used in the Box–Behnken Design

Type	Variable	Symbol	Low (-1)	Medium (0)	High (+1)	Goal
Factor	Sodium starch glycolate (mg)	A	XX	XX	XX	-
Factor	Microcrystalline cellulose (mg)	B	XX	XX	XX	-
Factor	PVP K30 (mg)	C	XX	XX	XX	-

Response	% Drug Release of Quercetin	Y_1	-	-	-	Maximize
Response	% Drug Release of Resveratrol	Y_2	-	-	-	Maximize
Response	Tablet Hardness (kg/cm²)	Y_3	-	-	-	Optimize
Response	Disintegration Time (min)	Y_4	-	-	-	Minimize

Pharmaceutical Evaluation of Optimized Formulations

The optimized quercetin–resveratrol immediate-release tablet formulations obtained through the Quality by Design (QbD) approach were evaluated to assess their pharmaceutical quality using standard pharmacopeial methods [30].

***In Vitro* Drug Release Study**

Drug release from the optimized immediate-release tablet formulations was evaluated using a USP Type II (paddle) dissolution apparatus [27–30]. The cumulative percentage drug release was calculated, and all experiments were carried out in triplicate. Results were expressed as mean ± standard deviation.

Sulforhodamine B (SRB) Assay

The antiproliferative activity of the optimized quercetin–resveratrol immediate-release tablet formulations was determined using the sulforhodamine B (SRB) assay against MCF-7 cells according to a reported procedure with slight modifications [33].

Statistical Analysis

All experiments were carried out in triplicate unless otherwise stated, and the results are presented as mean ± standard deviation. Statistical analysis of the optimization study was performed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). The optimization data were analysed using polynomial regression and response surface methodology implemented in Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, MN, USA). [24–26].

3. RESULTS

Molecular Docking of Selected Phytoconstituents against EGFR

The molecular docking scores of quercetin, resveratrol, naringenin, and the reference compound against EGFR are presented in Table 3. Among the investigated phytoconstituents, quercetin exhibited the highest binding affinity towards EGFR with a docking score of −9.0 kcal/mol, followed by naringenin (−8.2 kcal/mol) and resveratrol (−7.7 kcal/mol). The reference compound, gefitinib, exhibited a docking score of −8.5 kcal/mol. The two-dimensional protein–ligand interaction profiles of the investigated phytoconstituents and the reference compound with EGFR are shown in Figure 1.

Table 3. Molecular docking scores of phytoconstituents against EGFR (PDB ID: 1M17).

Compound	Binding affinity (kcal/mol)
Gefitinib	-8.5
Quercetin	-9.0
Resveratrol	-7.7
Naringenin	-8.2

Selection of Phytoconstituents (Quercetin and Resveratrol)

Based on the molecular docking results obtained against EGFR, quercetin, resveratrol, and naringenin demonstrated favourable binding affinities and were selected for subsequent in vitro cytotoxicity evaluation against MCF-7 breast cancer cells. Following biological assessment using the MTT assay, quercetin and resveratrol exhibited greater antiproliferative activity than naringenin. Consequently, quercetin and resveratrol were selected for further formulation development, whereas naringenin was excluded from the subsequent stages of the investigation.

***In-vitro* MTT Assay (Evaluation of Individual Phytoconstituents)**

The antiproliferative activity of quercetin, resveratrol, naringenin, and the reference drug gefitinib against MCF-7 breast cancer cells was evaluated using the MTT assay. All tested compounds exhibited concentration-dependent inhibition of cell viability, although differences in cytotoxic activity were observed among the investigated

phytoconstituents. Quercetin demonstrated the greatest antiproliferative activity among the phytoconstituents, with an IC_{50} value of 59.51 µg/mL, followed by resveratrol (67.05 µg/mL) and naringenin (78.22 µg/mL). As expected, gefitinib exhibited the highest cytotoxic activity with an IC_{50} value of 11.08 µg/mL.The IC_{50} values obtained for the investigated phytoconstituents are summarized in Table 4.Based on the observed antiproliferative activity, quercetin and resveratrol were selected for subsequent formulation development, whereas naringenin was excluded from further investigation.

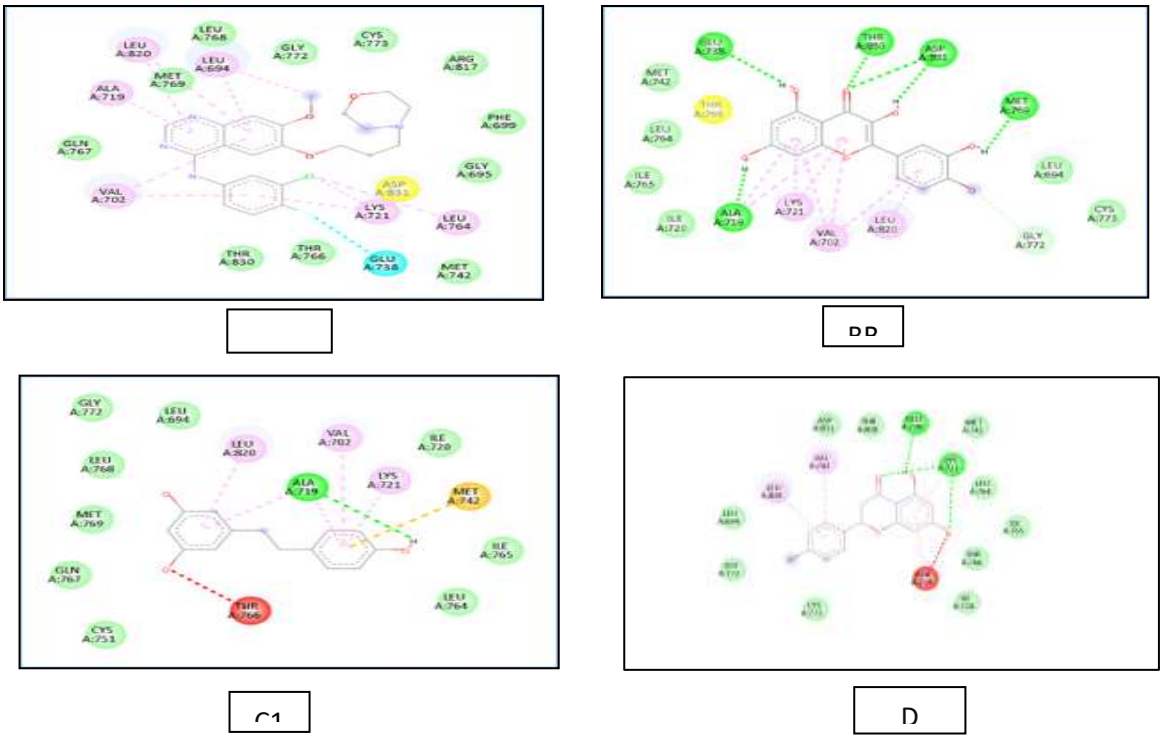


Figure 1. Two-dimensional interaction diagrams of the selected phytoconstituents and the reference compound with EGFR. (A) Gefitinib, (B) Quercetin, (C) Resveratrol, and (D) Naringenin.

Table 4. IC_{50} values of phytoconstituents and the reference drug against MCF-7 cells determined by the MTT assay.

Compound	IC_{50} (µg/mL)
Gefitinib	11.08
Quercetin	59.51
Resveratrol	67.05
Naringenin	78.22

Selection of Dose and Dosage Form

Based on the MTT assay results, quercetin and resveratrol were selected for further pharmaceutical development because of their comparatively greater antiproliferative activity against MCF-7 breast cancer cells. To investigate the influence of combination therapy, the selected phytoconstituents were incorporated into immediate-release tablet formulations containing quercetin and resveratrol in the ratios of 1:1, 1:2, and 2:1. Immediate-release tablets were selected as the dosage form for the present investigation because of their suitability for oral administration, rapid drug release characteristics, ease of manufacturing, and dose uniformity. The selected drug combinations were subsequently employed for formulation development and Quality by Design (QbD)-based optimization.

Formulation Development

Following the selection of quercetin and resveratrol, combination immediate-release tablet formulations were developed using the solid dispersion technique followed by direct compression. Solid dispersions of the selected phytoconstituents were prepared using polyvinylpyrrolidone K30 (PVP K30) as the solubility-enhancing carrier. The

resulting solid dispersions were blended with the required excipients and compressed into immediate-release tablets containing quercetin and resveratrol in the ratios of 1:1, 1:2, and 2:1. The developed formulations were uniform in appearance and were successfully compressed without visible defects such as capping, lamination, or chipping. The composition of the combination immediate-release tablet formulations is presented in Table 5. [27–30]

Table 5. Composition of Quercetin–Resveratrol Combination Immediate-Release Tablet Formulations.

Excipient	Functional role	Purpose in formulation
Quercetin Resveratrol	Active Pharmaceutical ingredient (API)	Anticancer activity
Microcrystalline cellulose (MCC)	Binder	Improves compressibility and tablet strength
Sodium Starch glycolate	Super-disintegrant	Facilitates rapid tablet disintegration
PVPK30	Solubility enhancer	Improves cohesion and mechanical strength
Mannitol	Diluent/ filler	Increases tablet bulk and improves mouth feel
Talc	Glident	Enhances powder flow
Magnesium stearate	Lubricant	Reduces friction during compression

Experimental Design and Formulation Batches

The quercetin–resveratrol combination immediate-release tablet formulations containing drug ratios of 1:1, 1:2, and 2:1 were optimized using a Quality by Design (QbD) approach based on a three-factor, three-level Box–Behnken Design (BBD). Sodium starch glycolate (SSG), microcrystalline cellulose (MCC), and polyvinylpyrrolidone K30 (PVP K30) were selected as the independent formulation variables, while percentage drug release of quercetin, percentage drug release of resveratrol, tablet hardness, and disintegration time were selected as the response variables [24–30]. The experimental design generated seventeen formulation batches with different combinations of the selected formulation variables. The observed responses obtained from all experimental runs are presented in Table 6.

Table 6. Experimental design matrix and observed responses of the Box–Behnken Design.

STD	Run	A: SSG (mg)	B: MCC (mg)	C: PVP K30(mg)	%Drug release of Quercetin	%Drug release of Resveratrol	DT (min)	Hardness (Kg/cm²)
17	1	19	157.5	20	74.98	68.56	1.24	7
13	2	19	157.5	20	75.00	67.20	1.27	7
5	3	18	157.5	15	73.46	66.87	1.27	6
12	4	19	165	25	69.50	67.89	1.32	7
1	5	18	150	20	74.34	71.80	1.27	6
4	6	20	165	20	72.64	67.45	1.31	7
10	7	19	165	15	72.56	66.65	1.34	7
3	8	18	165	20	73.23	67.87	1.43	7
6	9	20	157.5	15	76.86	66.45	1.21	5
15	10	19	157.5	20	75.87	68.20	1.32	6
14	11	19	157.5	20	75.88	67.98	1.30	6
11	12	19	150	25	76.23	71.20	1.27	5
7	13	18	157.5	25	74.38	68.89	1.29	6
9	14	19	150	15	75.80	69.23	1.23	4
2	15	20	150	20	76.32	72.23	1.20	5
8	16	20	157.5	25	74.98	71.80	1.23	6
16	17	19	157.5	20	76.80	71.00	1.24	5

Effect of Formulation Variables on Quercetin Drug Release

The percentage drug release of quercetin obtained from the experimental formulations ranged from 69.50% to 76.86%. Regression analysis demonstrated that the selected model adequately described the relationship between the formulation variables and quercetin drug release. The relationship between the independent variables and the response was represented by the following polynomial regression equation:

Equation (1):$\{\%\ Drug\ Release\ of\ Quercetin\} = 74.64 + 0.6738A - 1.84B - 0.4487C$

where A represents sodium starch glycolate (SSG), B represents microcrystalline cellulose (MCC), and C represents PVP K30.The regression coefficients indicate that increasing the concentration of SSG enhanced quercetin drug release, whereas increasing the concentrations of MCC and PVP K30 reduced the response within the investigated experimental range. Among the formulation variables, MCC exerted the greatest influence on quercetin drug release because of its comparatively larger regression coefficient. The combined effects of the formulation variables on quercetin drug release are illustrated in Figure 2 (A,B).

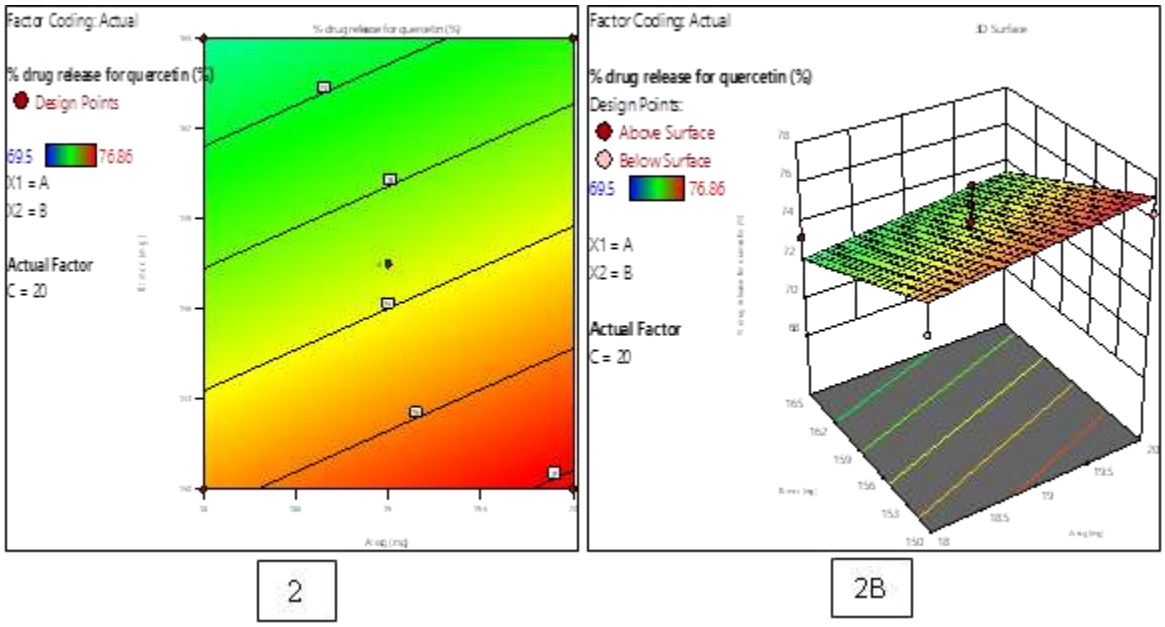


Figure 2. Effect of formulation variables on Quercetin drug release. (3A) Contour plot (3B) Three-dimensional response surface plot.

Effect of Formulation Variables on Resveratrol Drug Release

The percentage drug release of resveratrol among the experimental formulations ranged from 66.45% to 72.23%. Regression analysis adequately described the relationship between the formulation variables and resveratrol drug release. The relationship between the independent variables and the response was represented by the following polynomial regression equation:

Equation (2):$\{DrugReleaseofResveratrol(\%)\} = 68.90 + 0.3125A - 1.82B + 1.32C$

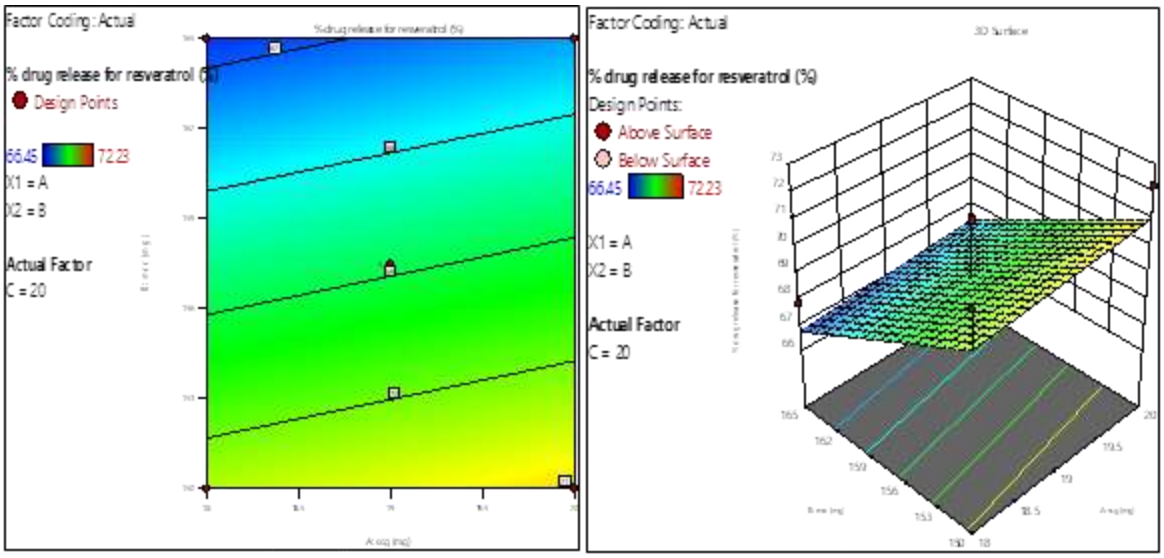
where A represents sodium starch glycolate (SSG), B represents microcrystalline cellulose (MCC), and C represents PVP K30.The positive regression coefficients of SSG and PVP K30 indicate that increasing their concentrations enhanced resveratrol drug release, whereas the negative coefficient of MCC indicates an inverse relationship with the response. Among the investigated variables, MCC exhibited the greatest influence on resveratrol drug release. The combined effects of the formulation variables on resveratrol drug release are illustrated in Figure 3 (A,B).

Effect of Formulation Variables on Disintegration Time

The disintegration time of the developed formulations ranged from 1.20 to 1.43 min. Regression analysis demonstrated that the selected model adequately represented the relationship between the formulation variables and tablet disintegration time. The relationship between the independent variables and the response was represented by the following polynomial regression equation:

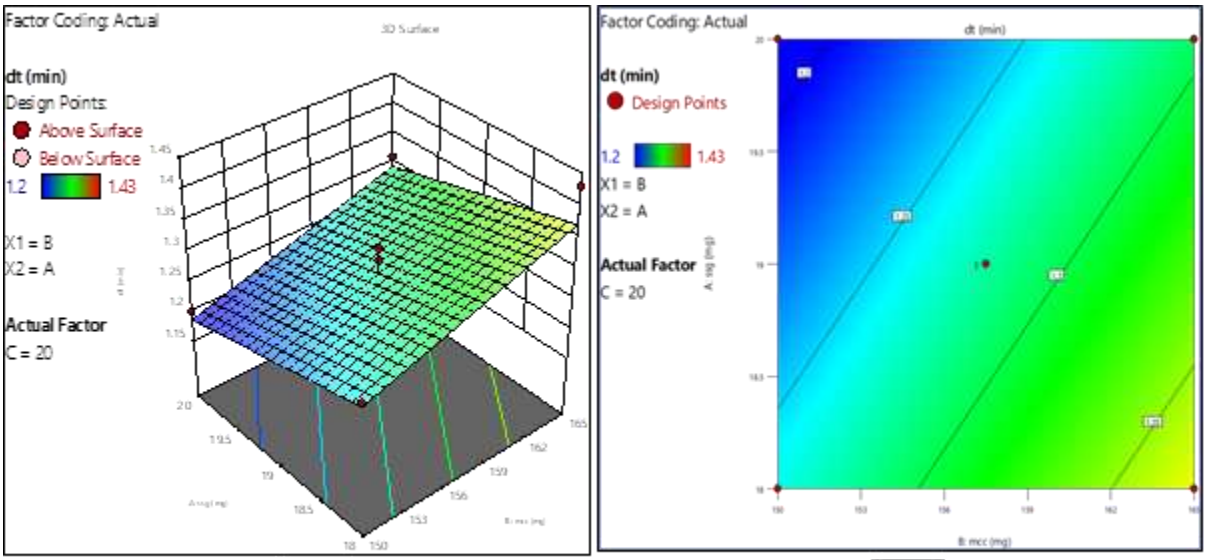
Equation (3):$\{Disintegration\ Time\ (min)\} = 1.28 - 0.0387A + 0.0538B + 0.0075C$

where A represents sodium starch glycolate (SSG), B represents microcrystalline cellulose (MCC), and C represents PVP K30.The regression coefficients indicate that increasing the concentration of SSG reduced tablet disintegration time, whereas increasing the concentrations of MCC and PVP K30 slightly increased the response. Among the investigated formulation variables, MCC exerted the greatest influence on tablet disintegration time. The combined effects of the formulation variables on tablet disintegration time are illustrated in Figure 4.



3A 3B

Figure 3 Effect of formulation variables on Resveratrol drug release. (4A) Contour plot (4B) Three-dimensional response surface plot.



4 4

Figure 4. Effect of formulation variables on Disintegration time.(4A) Contour plot(4B) Three- dimensional response surface plot.

Effect of Formulation Variables on Tablet Hardness

Tablet hardness values obtained from the experimental formulations ranged from 4 to 7 kg/cm². Regression analysis demonstrated that the selected model adequately represented the relationship between the formulation variables and tablet hardness. The relationship between the independent variables and the response was represented by the following polynomial regression equation:

Equation (4):$\left\{Hardness\left(\frac{kg}{cm^2}\right)\right\} = 6.00 - 0.2500A + 1.00B + 0.2500C$

where A represents sodium starch glycolate (SSG), B represents microcrystalline cellulose (MCC), and C represents PVP K30.The regression coefficients indicate that increasing the concentration of MCC enhanced tablet hardness, whereas increasing the concentration of SSG slightly reduced the response. PVP K30 also contributed positively to tablet hardness, although its influence was comparatively smaller than that of MCC. The combined effects of the formulation variables on tablet hardness are illustrated in Figure 5.

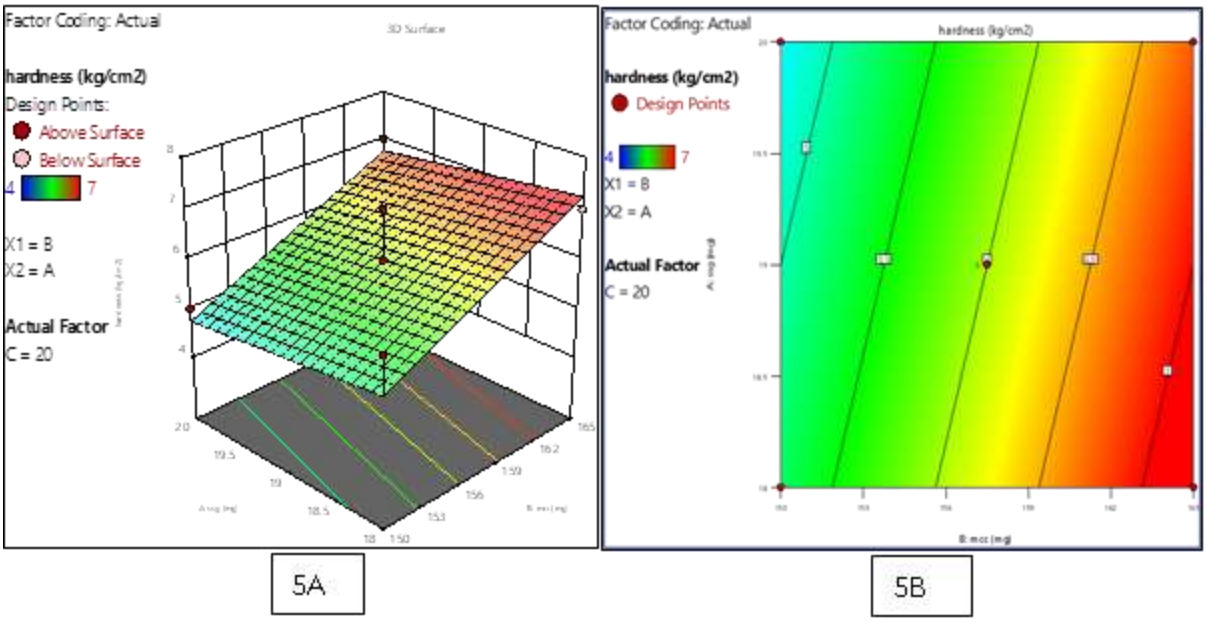


Figure 5. Effect of formulation variables on Tablet hardness.(6A) Contour plot(6B) Three-dimensional response surface plot

Validation of the Optimized Formulation

The optimized formulation predicted by the Design-Expert® software was prepared and evaluated to validate the optimization model. The experimentally obtained responses showed close agreement with the predicted values for percentage drug release of quercetin, percentage drug release of resveratrol, tablet hardness, and disintegration time. The comparison between the predicted and experimental values is presented in Table 7, confirming the reliability and predictive capability of the developed optimization model.

Table 7. Comparison of predicted and experimental responses of the optimized formulation.

Response	Predicted Value	Obtained value
Quercetin Release (%)	76.86	74.17
Resveratrol Release (%)	72.23	71.92
Disintegration Time (min)	1.19	1.19
Hardness (kg/cm²)	4.97	4.92

Pharmaceutical Evaluation

Ultraviolet–Visible Spectroscopic Analysis

Ultraviolet–visible spectroscopic analysis was performed to determine the wavelength of maximum absorbance (λmax) of quercetin and resveratrol prior to pharmaceutical evaluation. Quercetin exhibited a λmax at 372 nm, whereas resveratrol exhibited a λmax at 306 nm. These wavelengths were subsequently employed for calibration curve preparation, drug content estimation, and in vitro dissolution studies throughout the investigation [11,14].

Fourier Transform Infrared (FTIR) Analysis

Fourier transform infrared (FTIR) spectroscopy was performed to confirm the characteristic functional groups of quercetin and resveratrol. The recorded spectra exhibited the characteristic absorption bands of both phytoconstituents without any noticeable alterations, confirming their chemical identity. [11,14]

Pre-compression Evaluation

The obtained values indicated satisfactory flowability and compressibility of the powder blends for direct compression. The results of the pre-compression evaluation are presented in Table 8.

Table 8. Pre-compression evaluation of the optimized quercetin–resveratrol immediate-release tablet formulation.

Parameter	Result
Angle of Repose (°)	26.42 ± 0.84
Bulk Density (g/ml)	0.48 ± 0.01

Tapped Density (g/ml)	0.55 ± 0.01
Carr's Index (%)	12.73 ± 0.91
Hausner Ratio	1.15 ± 0.02

Post-compression Evaluation

The optimized immediate-release tablet formulation was evaluated for thickness, diameter, hardness, friability, average weight, and disintegration time. The prepared tablets exhibited uniform physical characteristics and complied with the acceptable pharmacopeial limits for the evaluated quality attributes. The results of the post-compression evaluation are summarized in Table 9.

Table 9. Post-compression evaluation of the optimized quercetin–resveratrol immediate-release tablet formulation.

Parameter	Result
Thickness (mm)	4.89 ± 0.16
Diameter (mm)	11.03 ± 0.06
Hardness (kg/cm²)	4.92 ± 0.24
Friability (%)	0.63
Average Weight (mg)	496.1 ± 4.9
Disintegration Time (min)	1.19 ± 0.04

In Vitro Drug Release Study

The formulation exhibited rapid and consistent drug release throughout the study period, demonstrating successful immediate-release performance for both phytoconstituents. The dissolution profile is presented in Figure 6.

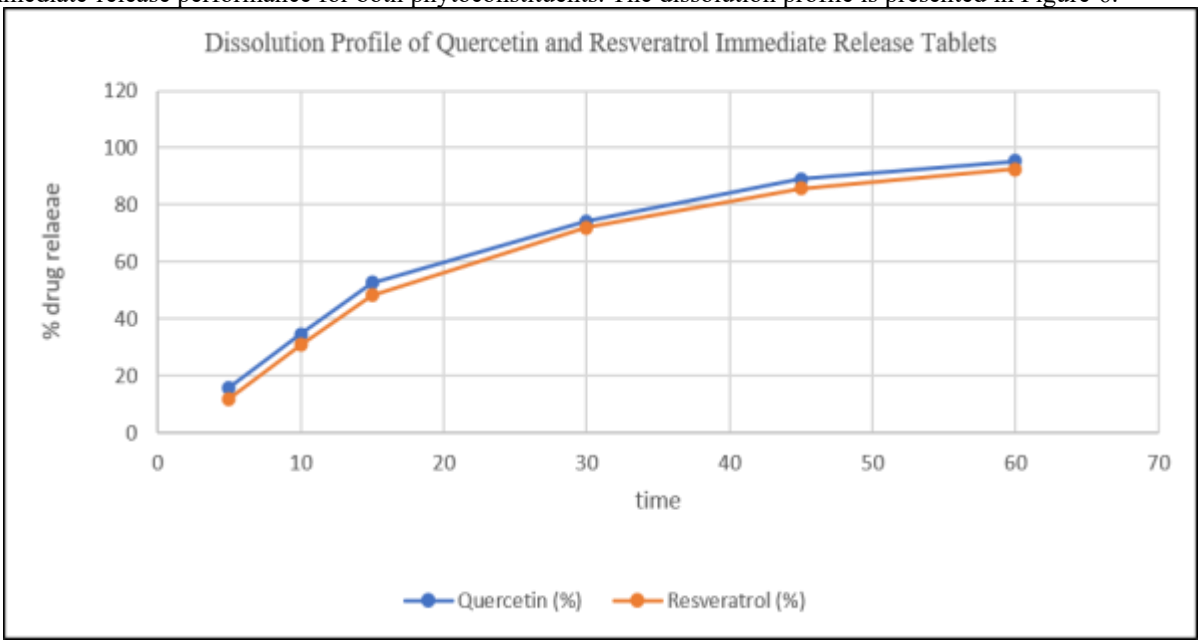


Figure 6. In vitro drug release profile of the optimized quercetin–resveratrol immediate-release tablet formulation.

Combination Immediate-Release Tablet Formulations for Biological Evaluation

Following pharmaceutical evaluation, the developed quercetin–resveratrol immediate-release tablet formulations containing drug ratios of 1:1, 1:2, and 2:1 were selected for subsequent biological evaluation against MCF-7 breast cancer cells. These formulations were subjected to the sulforhodamine B (SRB) assay to compare their antiproliferative activity and identify the formulation exhibiting the greatest cytotoxic potential. [27–30]

In-vitro Sulforhodamine B (SRB) Assay

The antiproliferative activity of the quercetin–resveratrol combination immediate-release tablet formulations containing drug ratios of 1:1, 1:2, and 2:1 was evaluated against MCF-7 breast cancer cells using the sulforhodamine B (SRB) assay. All formulations exhibited concentration-dependent inhibition of cell growth, although differences in cytotoxic activity were observed among the investigated formulations. The Quercetin: Resveratrol (2:1) formulation demonstrated the greatest antiproliferative activity with an IC_{50} value of <12.5 µg/mL, followed by the 1:1 formulation

(18.71 µg/mL) and the 1:2 formulation (22.18 µg/mL). The reference drug, gefitinib, exhibited an IC_{50} value of 11.08 µg/mL. The IC_{50} values of the investigated formulations are summarized in Table 10 [33].Representative microscopic observations of MCF-7 cells following treatment with the untreated control, gefitinib, and Quercetin: Resveratrol immediate-release tablet formulations (1:1, 1:2, and 2:1) are presented in Figure 7. Untreated control cells exhibited normal morphology and dense cell growth, whereas gefitinib-treated cells showed marked morphological alterations, including cell rounding, shrinkage, loss of adherence, and reduced cell density. Similar morphological changes were observed in cells treated with all Quercetin: Resveratrol formulations; however, the 2:1 formulation produced the most pronounced reduction in cell density and disruption of normal cellular morphology, followed by the 1:1 and 1:2 formulations. These qualitative microscopic observations were consistent with the quantitative SRB assay results and further supported the superior antiproliferative activity of the Quercetin: Resveratrol (2:1) formulation. Figure 7.

Table 10. IC_{50} values of quercetin–resveratrol combination immediate-release tablet formulations determined by the SRB assay.

Formulation	IC50 (µg/ml)
Gefitinib	11.08
Q: R (1:1)	18.71
Q: R (1:2)	22.18
Q: R (2:1)	<12.5

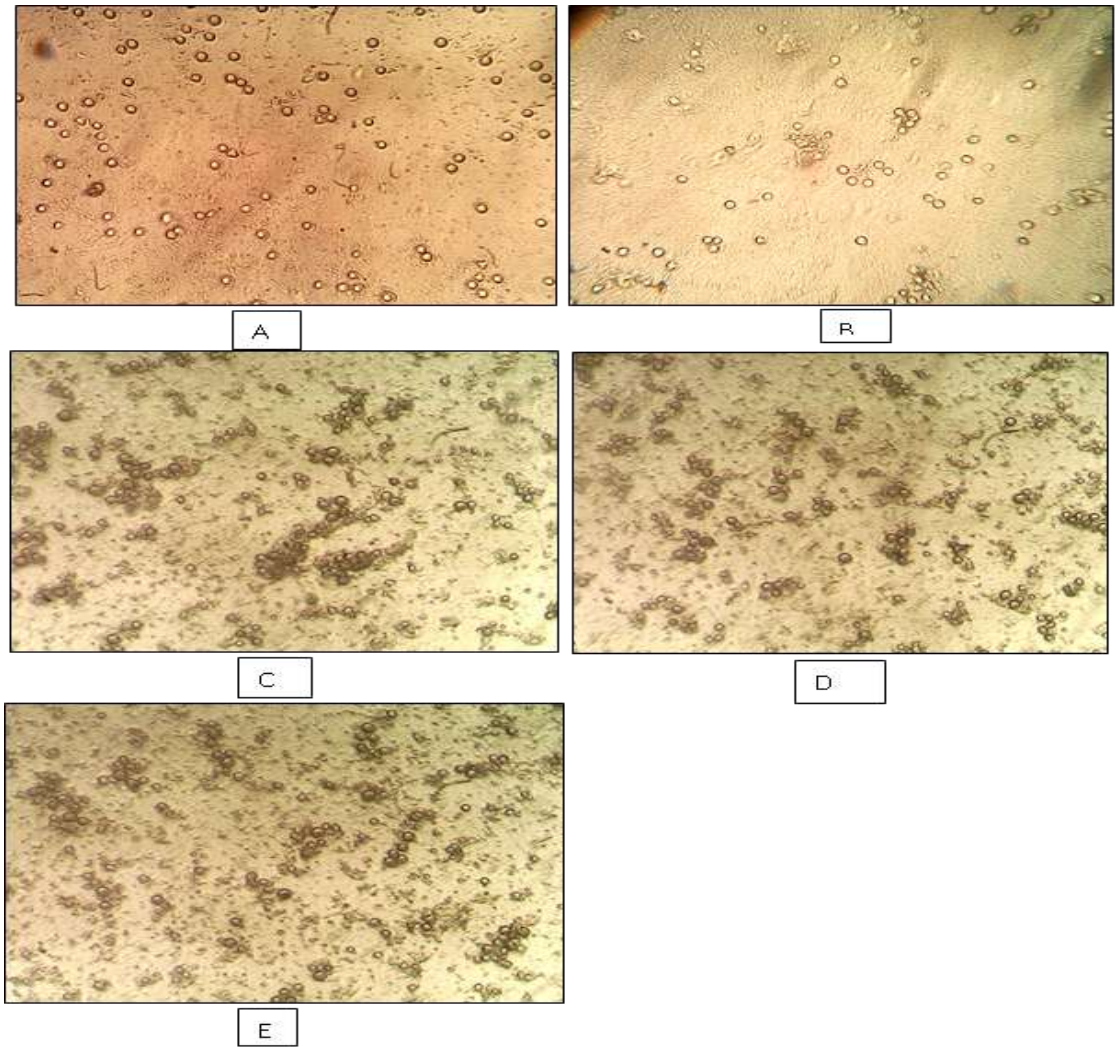


Figure 7. Microscopic observation of MCF-7 cells following treatment with (A) Untreated control, (B) Gefitinib (standard), (C) Quercetin: Resveratrol (1:1) immediate-release tablet formulation, (D)Quercetin: Resveratrol (1:2) immediate-release tablet formulation, and (E) Quercetin: Resveratrol (2:1) immediate-release tablet formulation.

Selection of the Optimized Quercetin: Resveratrol (2:1) Immediate-Release Tablet Formulation

Based on the combined findings of the SRB assay and microscopic evaluation, the Quercetin: Resveratrol (2:1) immediate-release tablet formulation demonstrated the greatest antiproliferative activity among the investigated combination formulations. It exhibited the lowest IC_{50} value (<12.5 µg/mL), which was comparable to that of the reference drug, gefitinib (11.08 µg/mL). In addition, microscopic examination revealed more pronounced morphological alterations in MCF-7 cells, including cell shrinkage, rounding, loss of adherence, and reduced cell density, compared with the 1:1 and 1:2 formulations. These findings confirmed the superior biological performance of the Quercetin: Resveratrol (2:1) immediate-release tablet formulation, which was therefore identified as the optimized formulation and selected for further investigation as a potential oral phytopharmaceutical for breast cancer management [33].

4. DISCUSSION

Molecular Docking against EGFR

As EGFR regulates tumour cell proliferation, survival, migration, and therapeutic resistance, its inhibition represents an established strategy for breast cancer treatment [5–10].Among the evaluated phytoconstituents, quercetin showed the highest binding affinity towards EGFR, followed by naringenin and resveratrol, indicating favourable receptor interactions. Although docking cannot confirm biological activity, it is an effective approach for prioritizing candidate compounds for experimental validation. Accordingly, the selected phytoconstituents were further evaluated using MCF-7 breast cancer cells. These findings are consistent with previous studies that have used molecular docking to identify phytoconstituents with potential anticancer activity before biological evaluation [5–10].

Selection of Phytoconstituents

Quercetin, resveratrol, and naringenin were initially shortlisted based on their favourable interactions with EGFR. Since molecular docking predicts binding affinity but cannot confirm biological activity, the selected compounds were further evaluated using the MTT assay against MCF-7 breast cancer cells. Based on the combined computational and biological findings, quercetin and resveratrol demonstrated superior antiproliferative activity and were selected for formulation development, whereas naringenin exhibited comparatively lower cytotoxic activity and was excluded. The selection of quercetin and resveratrol was further supported by their reported ability to modulate cell proliferation, apoptosis, oxidative stress, angiogenesis, and multiple signalling pathways involved in breast cancer progression. Their complementary mechanisms of action and favourable safety profiles also support their potential as candidates for combination therapy [11–22]. Overall, integrating molecular docking with in vitro screening enabled the rational selection of phytoconstituents with both favourable target interactions and measurable biological activity for subsequent formulation development.

***In-vitro* MTT Assay**

The MTT assay was performed to validate the antiproliferative activity of the selected phytoconstituents against MCF-7 breast cancer cells following molecular docking. While docking predicts ligand–protein interactions, cell-based assays are essential to confirm biological activity. Quercetin exhibited the highest cytotoxic activity, followed by resveratrol, whereas naringenin showed comparatively lower growth inhibition. These findings were consistent with the molecular docking results, where quercetin demonstrated the strongest binding affinity towards EGFR. Although resveratrol showed lower binding affinity than quercetin, its significant antiproliferative activity supported its selection as a combination partner. Previous studies have similarly reported that quercetin and resveratrol inhibit breast cancer cell proliferation through modulation of multiple signalling pathways, induction of apoptosis, and cell-cycle arrest [11–22]. Based on the combined computational and experimental findings, quercetin and resveratrol were selected for formulation development and combination studies.

Formulation Development

The formulation strategy was designed to overcome the poor aqueous solubility of quercetin and resveratrol, which can limit their dissolution and oral bioavailability. A solid dispersion approach using polyvinylpyrrolidone K30 (PVP K30) was employed to enhance the wettability, dispersibility, and dissolution of both phytoconstituents [27–30]. The resulting solid dispersions were then compressed into immediate-release tablets by the direct compression method. Direct compression was selected because of its simple manufacturing process, reduced processing steps, improved content uniformity, and minimal exposure of phytoconstituents to heat and moisture, thereby preserving their physicochemical stability. The combination of solid dispersion technology and direct compression provided an efficient approach for developing quercetin–resveratrol immediate-release tablets and established a suitable basis for subsequent Quality by Design (QbD)-based optimization [24–30].

Quality by Design (QbD)-Based Optimization
A Quality by Design (QbD) approach was employed to systematically optimize the quercetin–resveratrol immediate-release tablet formulation by evaluating the influence of critical formulation variables on the desired quality attributes. The Box–Behnken Design (BBD) enabled the simultaneous assessment of sodium starch glycolate (SSG), microcrystalline cellulose (MCC), and PVP K30, providing an efficient and scientific approach to formulation optimization while reducing the number of experimental runs [24–33].

***In-vitro* Sulforhodamine B (SRB) Assay**
Following formulation development and pharmaceutical evaluation, quercetin–resveratrol immediate-release tablet formulations containing different drug ratios (1:1, 1:2, and 2:1) were evaluated against MCF-7 breast cancer cells using the sulforhodamine B (SRB) assay. Unlike the MTT assay, which evaluated individual phytoconstituents, the SRB assay compared the antiproliferative activity of the formulated combination tablets. Among the investigated formulations, the Quercetin: Resveratrol (2:1) combination exhibited the greatest antiproliferative activity, with an IC_{50} value comparable to that of gefitinib, whereas the 1:1 and 1:2 formulations showed comparatively lower growth inhibition. These findings indicate that the drug ratio significantly influenced the biological activity of the developed formulations. The superior performance of the 2:1 formulation may be attributed to the complementary anticancer mechanisms of quercetin and resveratrol, including apoptosis induction, inhibition of cell proliferation, modulation of oxidative stress, and regulation of multiple signalling pathways involved in breast cancer progression, as reported in previous studies [11–22].

Significance of the Study
The present study employed a systematic approach integrating molecular docking, in vitro biological evaluation, formulation development, and Quality by Design (QbD)-based optimization to develop a phytoconstituent-based oral formulation for breast cancer management. Molecular docking and MTT assay enabled the rational selection of quercetin and resveratrol, while solid dispersion technology and Box–Behnken Design facilitated the development of an optimized immediate-release tablet with satisfactory pharmaceutical characteristics. Biological evaluation using the SRB assay further identified the Quercetin: Resveratrol (2:1) formulation as the most effective combination, exhibiting antiproliferative activity comparable to the reference drug, gefitinib. These findings demonstrate that integrating computational screening with experimental validation and pharmaceutical optimization provides a robust framework for developing phytoconstituent-based combination therapies. Although the optimized formulation showed promising in vitro performance, further pharmacokinetic, pharmacodynamic, stability, in vivo, and clinical studies are required to establish its therapeutic potential for breast cancer management.

5. CONCLUSION
The present study successfully developed and optimized a quercetin–resveratrol immediate-release tablet for potential breast cancer management through a systematic approach integrating computational screening, biological evaluation, pharmaceutical formulation, and Quality by Design (QbD)-based optimization. Molecular docking identified EGFR as the most suitable molecular target and facilitated the selection of promising phytoconstituents for further investigation. Subsequent in vitro MTT studies confirmed that quercetin and resveratrol exhibited greater antiproliferative activity than naringenin, supporting their selection for formulation development. Immediate-release tablet formulations containing different quercetin–resveratrol ratios were successfully prepared using a solid dispersion approach and optimized using the Box–Behnken Design.

REFERENCES
1. Chaurasia MC, Singh R, Sur S, Flora SJS. A review of FDA approved drugs and their formulations for the treatment of breast cancer. Front Pharmacol. 2023;14:1184472. doi:10.3389/fphar.2023.1184472.
2. Cardoso MJ, Poortmans P, Senkus E, Gentilini OD, Houssami N. Breast cancer highlights from 2023: knowledge to guide practice and future research. Breast. 2024;74:103674. doi:10.1016/j.breast.2024.103674.
3. Stordal B, Harvie M, Antoniou MN, Bellingham M, Chan DSM, Darbre P, et al. Breast cancer risk and prevention in 2024: an overview from the Breast Cancer UK–Breast Cancer Prevention Conference. Cancer Med. 2024;13:e70255. doi:10.1002/cam4.70255.
4. Uribe ML, Marrocco I, Yarden Y. EGFR in cancer: signaling mechanisms, drugs, and acquired resistance. Cancers (Basel). 2021;13(11):2748. doi:10.3390/cancers13112748.
5. Dutta S, et al. Advances in EGFR/HER2-directed clinical research on breast cancer. Adv Cancer Res. 2020;147:375-428. doi:10.1016/bs.acr.2020.04.009.
6. Sharma R, Singh P, Kumar A, et al. EGFR as therapeutic target to develop new generation tyrosine kinase inhibitors against breast cancer: A critical review. Results Chem. 2024;7:101490. doi:10.1016/j.rechem.2024.101490.

7. Azizan F, Sheriff RS, Goh CJH, Chiam KH, Koh CG. Solid stress compression enhances breast cancer cell migration through the upregulation of interleukin-6. Front Cell Dev Biol. 2025;13:1541953. doi:10.3389/fcell.2025.1541953.
8. Wang Y, et al. Can EGFR be a therapeutic target in breast cancer? BiochimBiophys Acta Rev Cancer. 2022;1877(5):188789. doi:10.1016/j.bbcan.2022.188789.
9. Duo J, Ying GG, Wang GW, Zhang L. Quercetin inhibits human breast cancer cell proliferation and induces apoptosis via Bcl-2 and Bax regulation. Mol Med Rep. 2012;5(6):1453-1456. doi:10.3892/mmr.2012.845.
10. Sharma R, Singh P, Kumar A, et al. EGFR as therapeutic target to develop new generation tyrosine kinase inhibitors against breast cancer: A critical review. Results Chem. 2024;7:101490. doi:10.1016/j.rechem.2024.101490.
11. Ezzati M, Yousefi B, Velaei K, Safa A. A review on anti-cancer properties of quercetin in breast cancer. Life Sci. 2020;248:117463. doi:10.1016/j.lfs.2020.117463.
12. Maugeri A, Calderaro A, Patanè GT, Navarra M, Barreca D, Cirmi S, et al. Targets involved in the anti-cancer activity of quercetin in breast, colorectal and liver neoplasms. Int J Mol Sci. 2023;24(3):2952. doi:10.3390/ijms24032952.
13. Fang L, Gao D, Wang T, Zhao H, Zhang Y, Wang S. From nature to clinic: quercetin's role in breast cancer immunomodulation. Front Immunol. 2024;15:1483459. doi:10.3389/fimmu.2024.1483459.
14. Brown K, Theofanous D, Aburido G, Pepper C, Howells L. Resveratrol for the management of human health: How far have we come? A systematic review of resveratrol clinical trials to highlight gaps and opportunities. Int J Mol Sci. 2024;25(2):747. doi:10.3390/ijms25020747.
15. Ahmad J, Algahtani MS, Garg A, Ahmad MZ, Imam SS. Nanotechnology-mediated delivery of resveratrol as promising strategy to improve therapeutic efficacy in triple negative breast cancer (TNBC): progress and promises. Expert Opin Drug Deliv. 2024;21(2):229-244. doi:10.1080/17425247.2024.2317194.
16. Talath S, El-Tanani M, Wali AF, et al. Natural supplements in breast cancer therapy: translational evidence, clinical safety, and emerging challenges. Front Pharmacol. 2026.
17. Alkharashi NA. Efficacy of resveratrol against breast cancer and hepatocellular carcinoma cell lines. Saudi Med J. 2023;44(3):254-261. doi:10.15537/smj.2023.44.3.20220768.
18. Fatehi R, Rashedinia M, Akbarizadeh AR, Zamani M, Firouzabadi N. Metformin enhances anti-cancer properties of resveratrol in MCF-7 breast cancer cells via induction of apoptosis, autophagy and alteration in cell cycle distribution. BiochemBiophys Res Commun. 2023;648:67-74. doi:10.1016/j.bbrc.2022.12.069.
19. Inchingolo AD, Inchingolo AM, Malcangi G, Avantario P, Azzollini D, Buongiorno S, et al. Effects of resveratrol, curcumin and quercetin supplementation on bone metabolism: a systematic review. Nutrients. 2022;14(17):3519. doi:10.3390/nu14173519.
20. Kumar D, Sharma PK. Quercetin: A comprehensive review. Curr Drug Metab. 2024;20(2):143-166. doi:10.2174/1573401319666230428152045.
21. Chen L, Wang Y, Zhang W, Liu X, Zhao H, Li J, et al. The effect of resveratrol, curcumin and quercetin combination on immuno-suppression of tumor microenvironment for breast tumor-bearing mice. Sci Rep. 2023;13(1):13768. doi:10.1038/s41598-023-39279-z.
22. Singh V, Singh R, Kujur PK, Singh RP. Combination of resveratrol and quercetin causes cell growth inhibition, DNA damage, cell cycle arrest, and apoptosis in oral cancer cells. Assay Drug Dev Technol. 2020;18(4):226-238. doi:10.1089/adt.2020.972.
23. Samaszko-Fiertek J, Dmochowska B, Madaj J. Anticancer activity of Schiff base metal complexes against MCF-7 breast cancer cell line. Int J Mol Sci. 2026;27(2):678. doi:10.3390/ijms27020678.
24. Reddy P, Kumar S, Sharma A. Optimization and Quality by Design approach for piroxicam fast dissolving tablet formulations using Box-Behnken design. Curr Drug Ther. 2020;15(2):152-165. doi:10.2174/1574885514666190409102614.
25. Ramireddy AR, Behara DK. QbD based formulation development and optimisation of ozenoxacin topical nano-emulgel and efficacy evaluation using impetigo mice model. AAPS PharmSciTech. 2024;25(5):90. doi:10.1208/s12249-024-02805-x.
26. Chen L, Wang J, Zhang Y. QbD-driven preparation, characterization, and pharmacokinetic study of nanoparticles. Sci Rep. 2025;15:85463. doi:10.1038/s41598-025-85463-8.
27. Yu LX. Pharmaceutical quality by design: product and process development, understanding, and control. Pharm Res. 2008;25(4):781-791. doi:10.1007/s11095-007-9511-1.
28. Palekar S, Barhate SD. Formulation optimization and evaluation of immediate release tablet of linagliptin. Int J Pharm Sci. 2025;3(6):2296-2308. doi:10.5281/zenodo.15645402.
29. Chavan S, Munnangi SR, Narala N, Vemula SK, Repka MA. Formulation and evaluation of immediate-release quetiapine fumarate tablets via twin-screw melt granulation. J Pharm Innov. 2025;20:65. doi:10.1007/s12247-025-09981-8.

30. Rej RK, Roy J, Allu SR. Therapies for the treatment of advanced/metastatic estrogen receptor-positive breast cancer: current situation and future directions. Cancers (Basel). 2024;16(3):552. doi:10.3390/cancers16030552.
31. Manikyam HK, Joshi SK, Vakadi S, Patil SB. Anticancer activity of novel terpenoid saponin of Psidium guajava on MCF-7 cancer cell line using DAPI and MTT assays. Afr J Pharm Pharmacol. 2021;15(12):206-211.
32. Ozalp Y, Hourani OM, Chunu JT, Yegen G, Aksu NB. Optimization of metformin hydrochloride tablet by direct compression method using Quality by Design approach. Indian J Pharm Sci. 2023;85(4):1015-1024. doi:10.36468/pharmaceutical-sciences.1168.
33. Zaluzec EK, Sempere LF. Systemic and local strategies for primary prevention of breast cancer. Cancers (Basel). 2024;16(2):248. doi:10.3390/cancers16020248.