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Cytotoxic Activity of *Plumbago zeylanica* Chloroform Extract Against T47D Breast Cancer Cells: An Integrated In Vitro and In Silico Study

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Abstract

Natural products remain a valuable source of anticancer agents due to their chemical diversity and multiple biological activities. Although *Plumbago zeylanica* has been widely recognized as a medicinal plant, the phytochemical composition and anticancer mechanism of its chloroform leaf extract against T47D breast cancer cells remain insufficiently characterized. This study aimed to investigate the phytochemical profile, total phenolic and flavonoid contents, cytotoxic activity, and potential molecular mechanism of the chloroform leaf extract through an integrated in vitro and in silico approach. Phytochemical constituents were characterized by GC–MS, total phenolic and flavonoid contents were determined spectrophotometrically, cytotoxic activity was evaluated using the MTT assay, and molecular docking was performed against the anti-apoptotic Bcl-2 protein. GC–MS analysis identified β-sitosterol (22.65%), *n*-hexadecanoic acid (16.84%), cis-vaccenic acid (12.51%), phytol (5.37%), and stigmasterol (4.34%) as the major constituents. The extract exhibited high total phenolic (99.60 mg GAE/g extract) and flavonoid (69.67 mg QE/g extract) contents, together with moderate cytotoxic activity against T47D cells (IC_{50} = 36.34 µg/mL). Molecular docking revealed that stigmasterol showed the strongest binding affinity toward Bcl-2 (ΔG = −6.6 kcal/mol), suggesting a potential contribution to apoptosis-related activity. Collectively, these findings indicate that the chloroform leaf extract of *P. zeylanica* contains bioactive phytochemicals with moderate antiproliferative activity against T47D breast cancer cells and provides a basis for further mechanistic and preclinical investigations.

Keywords: Breast cancer; Cytotoxicity; Molecular docking; *Plumbago zeylanica*; T47D cells.

INTRODUCTION

Breast cancer remains the most frequently diagnosed malignancy and one of the leading causes of cancer-related mortality among women worldwide, with approximately 2.3 million new cases and more than 660,000 deaths reported annually (Bray et al., 2024). Although substantial advances have been achieved in surgery, chemotherapy, radiotherapy, endocrine therapy, and targeted therapy, the effectiveness of these treatments is often limited by multidrug resistance, tumor recurrence, tumor heterogeneity, and systemic toxicity (Barrios, 2022; Hasan-Abad et al., 2024). Consequently, the discovery of safer and more effective anticancer agents from natural sources has become an important area of pharmaceutical research. Plant-derived secondary metabolites have attracted considerable attention because of their structural diversity and their ability to modulate multiple signaling pathways associated with cancer progression, including apoptosis, angiogenesis, autophagy, and cell-cycle regulation (Cragg & Newman, 2013; Papadopoulou et al., 2026).

Among medicinal plants, *P. zeylanica* (Plumbaginaceae) has long been used in traditional medicine throughout Asia and Africa for the treatment of inflammation, fever, pain, gastrointestinal disorders, and other ailments (Jose & Panneerselvam, 2019; Pant et al., 2012; Singh et al., 2017). Phytochemical studies have revealed that this species contains diverse bioactive constituents, including naphthoquinones, phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, and fatty acids, many of which possess antioxidant and anticancer activities (Purwoko et al., 2022; Shukla et al., 2021). Among these compounds, plumbagin has been extensively investigated and has demonstrated potent antiproliferative activity against breast cancer cells through apoptosis induction, cell-cycle arrest, modulation of reactive oxygen species, and inhibition of signaling pathways such as PI3K/Akt, NF-κB, STAT3, and MAPK (Sastri et al., 2026). However, the pharmacological activity of crude plant extracts cannot be attributed solely

to plumbagin, as interactions among multiple co-occurring phytochemicals may produce synergistic or additive effects that contribute to the overall biological activity.

The biological activity of plant extracts is highly dependent on the extraction solvent because solvent polarity determines the classes of metabolites recovered (Dirar et al., 2019). Chloroform, a semi-polar solvent, has been reported to efficiently extract lipophilic compounds, including naphthoquinones and other metabolites associated with cytotoxic activity (Devi et al., 2021). Nevertheless, information regarding the cytotoxic activity of the chloroform leaf extract of *P. zeylanica* against T47D human breast cancer cells remains limited. T47D cells represent an estrogen receptor-positive (ER-positive) breast cancer subtype that overexpresses the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein, which suppresses mitochondrial apoptosis and contributes to tumor survival and therapeutic resistance (Fairlie & Lee, 2021; Hafezi & Rahmani, 2021). Since apoptosis induction is a major mechanism underlying the anticancer effects of many natural products, Bcl-2 represents an attractive molecular target for investigating the potential mechanism of action of phytochemicals present in *P. zeylanica* (Rajabi et al., 2021; Yuan et al., 2022).

Comprehensive characterization of crude extracts is essential for identifying the phytochemicals responsible for their biological activity. Gas chromatography–mass spectrometry (GC–MS) is widely employed for profiling volatile and semi-volatile phytochemicals in plant extracts because of its high sensitivity, reproducibility, and analytical accuracy (Dirar et al., 2019). Molecular docking is a valuable *in silico* approach for predicting ligand–protein interactions and estimating the binding affinity of bioactive compounds toward molecular targets associated with cancer progression (Ferreira et al., 2015; Pinzi & Rastelli, 2019). Therefore, integrating GC–MS-based phytochemical profiling with molecular docking provides complementary evidence for interpreting cytotoxic activity and identifying candidate bioactive compounds for further pharmacological investigation (Ene et al., 2026).

Despite increasing interest in the anticancer potential of *P. zeylanica*, several important knowledge gaps remain. Most previous studies have focused on isolated plumbagin rather than crude chloroform leaf extracts, leaving the contribution of other phytochemicals and their possible synergistic effects largely unexplored (Purwoko et al., 2022; Sastri et al., 2026; Shukla et al., 2021). In addition, the cytotoxic activity of the chloroform leaf extract against ER-positive T47D breast cancer cells has not been comprehensively evaluated, and no previous study has integrated GC–MS-based phytochemical profiling with molecular docking targeting the anti-apoptotic Bcl-2 protein to provide preliminary mechanistic insight into the extract's cytotoxic activity.

Therefore, this study aimed to evaluate the cytotoxic activity of the chloroform leaf extract of *P. zeylanica* against T47D human breast cancer cells, characterize its phytochemical constituents using GC–MS, and investigate the interactions of the major identified compounds with the Bcl-2 protein through molecular docking. By integrating *in vitro* cytotoxic evaluation, phytochemical characterization, and *in silico* analysis, this study is expected to provide a more comprehensive understanding of the anticancer potential of *P. zeylanica* and identify promising phytochemicals for further mechanistic and pharmacological studies.

MATERIALS AND METHODS

Materials

The materials included *P. Zeylanica* leaves, chloroform, Folin–Ciocalteu reagent, gallic acid, quercetin, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, and distilled water. Reagents used for the cytotoxicity assay comprised RPMI and DMEM culture media, phosphate-buffered saline (PBS), 0.25% trypsin–EDTA, dimethyl sulfoxide (DMSO), 10% sodium dodecyl sulfate (SDS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), and T47D human breast cancer cells. The study employed standard laboratory equipment, including glassware, a maceration apparatus, rotary evaporator, UV–Vis spectrophotometer (Hitachi U5100 Double Beam, Japan), analytical balance, oven, hotplate stirrer, blender, micropipettes, and a 60-mesh sieve. Cytotoxicity assays were performed using 96-well microplates, a microplate ELISA reader (BIO-RAD Benchmark, USA), a 5% CO_2 incubator (NAPCO Model 6200, Heraeus, Germany), an inverted microscope (Olympus CKX41, Japan), a Class II biosafety cabinet, and a hemocytometer.

Sampel preparation and extraction

Fresh leaves of *P. zeylanica* were washed, cut into small pieces, and air-dried under sunlight until a constant weight was achieved. The dried leaves were ground into a fine powder, and the dry weight was recorded. The powdered material was extracted with chloroform by maceration at a 1:10 (w/v) ratio for 72 h at room temperature, with the solvent replaced every 24 h. The combined filtrates were filtered and concentrated under reduced pressure using a rotary evaporator to obtain the crude chloroform extract.

Total Phenolic Content (TPC)

The total phenolic content (TPC) of the chloroform extract was determined using the Folin–Ciocalteu colorimetric method according to Pranoothi et al., (2014) with slight modifications. Briefly, 5 mg of the extract

was dissolved in 10 mL of distilled water. An aliquot of 1 mL of the sample solution was mixed with 5 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent. After 5 min, 1.5 mL of 20% sodium carbonate solution was added, and the mixture was incubated in the dark at room temperature for 60 min. The absorbance was then measured at 765 nm using a UV–Vis spectrophotometer, with distilled water used as the blank. Gallic acid standard solutions (20–100 µg/mL) were used to construct the calibration curve. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per g dry weight (DW) of the extract.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the chloroform extract was determined using the aluminum chloride colorimetric method described by Beyene et al., (2020) with slight modifications. Briefly, 1 mL of the extract or quercetin standard solution (10–50 µg/mL) was mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 min, 0.3 mL of 10% aluminum chloride solution was added, and the mixture was incubated at room temperature in the dark for 6 min. Subsequently, 2 mL of 1 M sodium hydroxide was added, and the absorbance was measured at 507 nm using a UV–Vis spectrophotometer, with distilled water used as the blank. The total flavonoid content was calculated from the quercetin calibration curve and expressed as mg quercetin equivalents (QE) per 100 g dry weight (DW).

GC-MS Analysis

The resulting extract was subsequently analyzed by gas chromatography–mass spectrometry (GC–MS) to identify its chemical constituents prior to biological evaluation.

Cytotoxicity Assay

Cytotoxic activity against T47D breast cancer cells was evaluated using the MTT assay according to Yousef et al., (2018) with minor modifications. Briefly, confluent cells were seeded into 96-well plates and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. The culture medium was replaced with 100 µL of serially diluted sample solutions, followed by incubation for another 24 h. After treatment, the medium was removed, and 100 µL of MTT solution was added to each well. The plates were incubated for 4 h to allow formazan formation. Subsequently, 100 µL of 10% SDS in 0.1 M HCl was added to dissolve the formazan crystals, and the plates were incubated overnight in the dark at room temperature. Absorbance was measured at 595 nm using a microplate reader. Cell viability (%) was calculated using the following equation:

$$\text{Cell Viability (\%)} = \frac{A_T - A_{blank}}{A_S - A_{blank}} \times 100$$

where A_S is the absorbance of untreated control cells, A_T is the absorbance of treated cells, and A_{blank} is the absorbance of the medium blank. Cell inhibition (%) was calculated as $\mathbf{100} -$ Cell Viability (%), and IC_{50} values were determined from the dose–response curves.

Molecular docking

Molecular docking simulations were conducted to investigate the potential interactions between the major phytochemical constituents of the chloroform extract of *P. zeylanica* and the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), with particular emphasis on their interactions with amino acid residues within the protein binding site. The three-dimensional structure of Bcl-2 (PDB ID: 2W3L) was retrieved from the Protein Data Bank, and docking calculations were performed using AutoDock Vina version 1.5.7. The co-crystallized ligand was used to define the binding site and validate the molecular docking. The molecular docking was validated by re-docking the co-crystallized ligand into the Bcl-2 binding site. The grid box was set to 20 × 20 × 20 Å and centered at x = 28.519, y = 41.812, and z = 6.296. Docking accuracy was assessed using the root-mean-square deviation (RMSD) between the crystallographic and re-docked ligand poses, with values <2.0 Å considered acceptable (Trott & Olson, 2010).

The five major compounds identified by GC–MS, namely stigmasterol, β-sitosterol, phytol, *n*-hexadecanoic acid (palmitic acid), and *cis*-vaccenic acid, were subsequently docked into the Bcl-2 binding site using the validated procedure. The predicted binding free energy (ΔG) and inhibition constant (Ki) were used to compare the predicted ligand–Bcl-2 interactions. The resulting complexes were analyzed based on hydrogen-bonding, hydrophobic, and van der Waals interactions. Two-dimensional and three-dimensional interaction profiles were generated using Discovery Studio Visualizer 2019, whereas RMSD calculations and structural superposition analyses were performed using PyMOL.

RESULTS AND DISCUSSION

Extraction Yield

The powdered leaves of *P. zeylanica* were extracted with chloroform by maceration for 72 h at room temperature, with the solvent replaced every 24 h to improve the extraction efficiency (Azwanida, 2015; Sasidharan et al., 2011). Chloroform was selected as the extraction solvent because its semi-polar to non-polar characteristics enable the efficient extraction of lipophilic constituents, while extraction efficiency is largely governed by the compatibility between solvent polarity and the target phytochemicals (Kamari et al., 2024; Popova & Bankova, 2023). The extraction of 100 g of dried *P. zeylanica* leaf powder yielded 4.68% (w/w) crude extract. This yield indicates that chloroform effectively recovered the semi-polar and non-polar secondary

metabolites present in *P. zeylanica* leaves. Extraction yield is influenced by several factors, including solvent type, solvent polarity, extraction time, solvent-to-solid ratio, particle size, and the physicochemical characteristics of the plant. Although more polar solvents generally produce higher extraction yields, chloroform provides greater selectivity toward lipophilic bioactive compounds that are frequently associated with pharmacological activity.

Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of the chloroform extract of *P. zeylanica* leaves are presented in Table 1.

Table 1. Total phenolic and total flavonoid content of the chloroform extract of *P. zeylanica* leaves.

Parameter	Content
Total phenolic content (TPC)	99,60 mg GAE/g dry extract
Total flavonoid content (TFC)	69,67 mg QE/g dry extract

The extract contained 99.60 mg GAE/g dry extract of total phenolics and 69.67 mg QE/g dry extract of total flavonoids, indicating that the chloroform extract is a rich source of phenolic and flavonoid compounds. Phenolic compounds and flavonoids are major classes of plant secondary metabolites that play important roles in plant defense and exhibit diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. Their antioxidant activity is mainly associated with their ability to scavenge reactive oxygen species (ROS), donate electrons or hydrogen atoms, and regulate oxidative stress-related signaling pathways (Al-Nablsi et al., 2022; Ghasemzadeh & Ghasemzadeh, 2011). Therefore, the relatively high TPC and TFC values observed in the present study suggest that the chloroform extract possesses considerable antioxidant potential, which may contribute to its biological activity. The high recovery of phenolic and flavonoid compounds may be attributed to the semipolar nature of chloroform. Solvent polarity is one of the most important factors influencing extraction efficiency because it determines the solubility of phytochemicals with different polarities. Semipolar solvents such as chloroform are capable of extracting moderately polar phenolic compounds and flavonoids while minimizing the extraction of highly polar constituents (Sun et al., 2025). Consequently, the extraction conditions employed in this study appear to be suitable for recovering a substantial amount of these bioactive metabolites.

The TPC and TFC values obtained in the present study were comparable to those previously reported for *P. zeylanica* leaf extracts, although some variations were observed depending on the extraction solvent. Beyene et al., (2020) reported TPC values of 51.63, 69.78, and 82.74 mg GAE/g dry extract for petroleum ether, ethyl acetate, and methanolic extracts, respectively, while the TFC value obtained in this study (69.67 mg QE/g dry extract) was comparable to that of the methanolic extract (69.57 mg QE/g dry extract) and slightly higher than those of petroleum ether and ethyl acetate extracts. These differences are likely associated with the polarity of the extraction solvent, which affects the recovery of phenolic and flavonoid compounds. A recent metabolomic study by Zeng et al., (2024) also reported that *P. zeylanica* leaves extracts contained 54.66 ± 0.80 mg GAE/g DW of TPC and 94.12 ± 1.07 mg RE/g DW of TFC, further demonstrating that the phytochemical content of the leaves varies among studies. Such variations are expected because phenolic and flavonoid contents are influenced by several factors, including extraction solvent, extraction conditions, geographical origin, plant maturity, and analytical methods. Overall, the relatively high TPC and TFC values obtained in the present study support the suitability of chloroform as a semipolar solvent for extracting phenolic and flavonoid compounds from *P. zeylanica* leaves.

GC-MS Analysis

GC–MS analysis was performed to characterize the phytochemical constituents present in the chloroform leaf extract of *Plumbago zeylanica*. The total ion chromatogram (TIC) obtained from the GC–MS analysis is presented in Figure 1, illustrating the separation of volatile and semi-volatile constituents according to their retention times. The chromatogram exhibited numerous well-resolved peaks with varying intensities, indicating the chemical complexity of the extract.

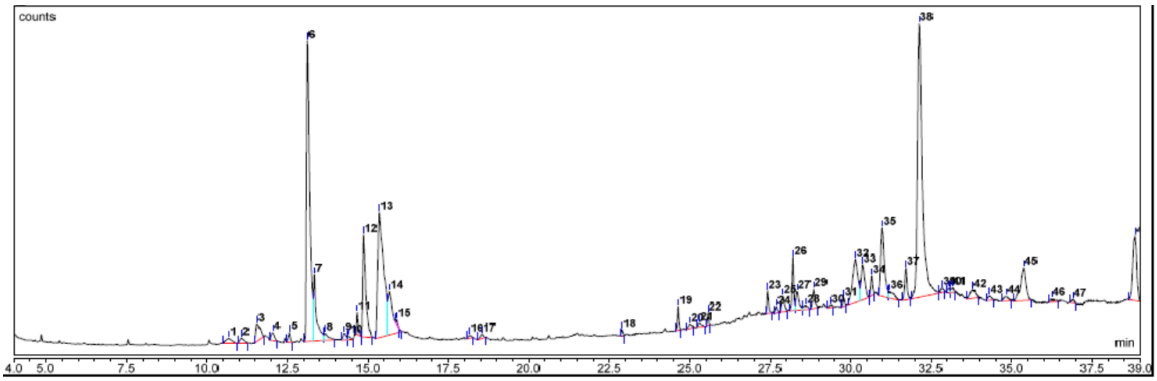


Figure 1. Total ion chromatogram (TIC) of the chloroform leaf extract of *P. zeylanica* obtained by GC–MS analysis.

Based on comparison of the mass spectra with the NIST library, 48 phytochemical compounds were identified. The complete list of compounds, together with their retention times, molecular formulas, molecular weights, and relative peak areas, is presented in Table 2. The identified constituents were predominantly phytosterols, diterpenoids, fatty acids, and their derivatives.

Table 2. Five major compounds identified in the chloroform extract of *P. zeylanica* leaves by GC–MS analysis.

No.	Retention Time (min)	Compounds	Chemical Formula	Molecular Weight (g/mol)	Peak Area (%)	Reported biological activities	References
1	32.13	β-Sitosterol	$C_{29}H_{50}O$	414.71	22.65	Antioxidant, anti-inflammatory, antidiabetic, anticancer, antibacterial, immunomodulatory, hypocholesterolemic, and hepatoprotective activities	(Babu & Jayaraman, 2020; Durrani et al., 2024; Khan & Siddiqui, 2020; Nandi et al., 2024; Wang et al., 2023)
2	13.11	n-Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.42	16.84	Antimicrobial, anti-inflammatory, and antioxidant	(Bashir et al., 2024; Purushothaman et al., 2025)
3	15.35	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	12.51	Antioxidant, anti-inflammatory, antibacterial, and lipid metabolism-regulating activities	(Raina et al., 2025)
4	14.86	Phytol	$C_{20}H_{40}O$	296.53	5.37	Antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, anxiolytic, and immunostimulatory activities	(Duraisamy et al., 2024; Sakthivel et al., 2018)
5	30.98	Stigmasterol	$C_{29}H_{48}O$	412.69	4.34	Antioxidant, anti-inflammatory, antidiabetic, anticancer, hypocholesterolemic, antifungal, and immunomodulatory activities	(Bakrim et al., 2022)

Among the identified compounds, β-sitosterol (22.65%) was the predominant constituent, followed by n-hexadecanoic acid (16.84%), cis-vaccenic acid (12.51%), phytol (5.37%), and stigmasterol (4.34%). These five compounds accounted for a substantial proportion of the extract composition and were therefore selected for further discussion. Previous studies have reported that these metabolites possess antioxidant, anti-inflammatory, and anticancer activities, suggesting that they may contribute to the cytotoxic activity of the chloroform extract against T47D breast cancer cells.

Cytotoxic Activity of the Chloroform Extract Against T47D Breast Cancer Cells

The chloroform leaf extract of *P. zeylanica* exhibited cytotoxic activity against T47D human breast cancer cells with an IC_{50} value of 36.34 µg/mL, whereas cisplatin, used as the positive control, showed a significantly lower IC_{50} value of 12.28 µg/mL (Table 3). These findings indicate that although the extract was less potent than cisplatin, it effectively inhibited the viability of T47D cells. According to the National Cancer Institute (NCI) criteria, crude plant extracts with IC_{50} values between 20 and 100 µg/mL are classified as moderately cytotoxic, indicating that the chloroform extract possesses promising antiproliferative activity and merits further investigation as a potential source of anticancer compounds.

Table 3. Cytotoxic activity of the chloroform extract of *P. zeylanica* leaves against T47D breast cancer cells.

Sample	IC_{50} (µg/mL)
Chloroform extract	36.340
Cisplatin (positive control)	12.284

The observed cytotoxic activity is likely associated with the phytochemical composition of the chloroform extract. As shown by the GC–MS analysis (Figure 1 and Table 2), the extract contained 48 phytochemical constituents, predominantly phytosterols, diterpenoids, and fatty acids. Among these compounds, β-sitosterol (22.65%), n-hexadecanoic acid (16.84%), cis-vaccenic acid (12.51%), phytol (5.37%), and stigmasterol (4.34%) were identified as the major constituents. The abundance of these metabolites suggests that the cytotoxic activity observed against T47D cells is unlikely to result from a single compound but rather from the combined action of multiple bioactive constituents present in the extract. Among the identified phytochemicals, β-sitosterol, which constituted the largest proportion of the extract (22.65%), has been extensively reported to exhibit anticancer activity against breast cancer cells. Previous studies demonstrated that β-sitosterol suppresses cancer cell proliferation by inducing apoptosis through activation of caspase-3 and caspase-9, increasing the Bax/Bcl-2 ratio, and inhibiting the PI3K/Akt signaling pathway,

ultimately leading to mitochondrial dysfunction and programmed cell death (Zhu et al., 2025). The high abundance of β-sitosterol in the present extract therefore suggests that this phytosterol may contribute substantially to the observed cytotoxic activity against T47D cells.

In addition to β-sitosterol, stigmasterol (4.34%) has also been reported to induce apoptosis in breast cancer cells by modulating mitochondrial membrane permeability, increasing reactive oxygen species production, and regulating apoptosis-related proteins such as Bax and Bcl-2 (Li et al., 2025).

Likewise, phytol (5.37%), an acyclic diterpene alcohol identified in the extract, possesses antiproliferative activity through induction of oxidative stress, disruption of mitochondrial membrane potential, activation of caspases, and cell-cycle arrest (Sakthivel et al., 2018). These findings suggest that phytosterols and diterpenoids present in the chloroform extract may act cooperatively to suppress breast cancer cell growth.

The extract also contained relatively high levels of n-hexadecanoic acid (palmitic acid) and cis-vaccenic acid, accounting for 16.84% and 12.51% of the total chromatographic area, respectively. Although these fatty acids generally exhibit weaker cytotoxic activity than phytosterols, previous studies have suggested that long-chain fatty acids may enhance membrane permeability, influence lipid metabolism, and modulate intracellular signaling pathways associated with apoptosis. Furthermore, fatty acids may facilitate the uptake or biological activity of other phytochemicals present in crude extracts, thereby contributing to additive or synergistic anticancer effects (Akasha et al., 2025; Budi et al., 2022). Consequently, the moderate cytotoxic activity observed in the present study may result from synergistic interactions among phytosterols, diterpenoids, and fatty acids rather than the action of an individual compound.

Although the cytotoxic activity of the chloroform extract was lower than that of cisplatin, the extract demonstrated promising antiproliferative potential against T47D cells. Unlike cisplatin, which is a single purified compound, crude plant extracts contain multiple phytochemicals that may exert biological activity through additive or synergistic interactions. In the present study, β-sitosterol, stigmasterol, phytol, diterpenoids, and long-chain fatty acids were identified as the major metabolites. Previous studies have shown that these compounds possess anticancer properties by inhibiting cell proliferation, inducing apoptosis, and modulating cancer-related signaling pathways. Therefore, the cytotoxic activity observed in this study is likely the combined effect of these bioactive constituents rather than the action of a single compound.

The MTT assay confirmed that the chloroform extract reduced the viability of T47D breast cancer cells; however, it does not reveal the underlying mechanism of cell death. Therefore, molecular docking was performed using the major phytochemicals identified by GC–MS against the anti-apoptotic Bcl-2 protein. Since Bcl-2 plays a crucial role in regulating the intrinsic apoptotic pathway in ER-positive breast cancer cells, the docking analysis provides preliminary evidence of the potential interactions between the identified phytochemicals and this therapeutic target. Overall, the integration of cytotoxicity evaluation, GC–MS phytochemical profiling, and molecular docking suggests that the chloroform leaf extract of *P. zeylanica* possesses promising antiproliferative activity against T47D breast cancer cells. The abundance of phytosterols, phytol, diterpenoids, and other bioactive metabolites provide a plausible phytochemical basis for the observed cytotoxicity, while molecular docking offers preliminary insight into their potential mechanism through Bcl-2 inhibition. These findings support further studies to validate the anticancer potential of *P. zeylanica* through mechanistic investigations and in vivo evaluation.

Molecular Docking Analysis

Molecular docking analysis was performed to investigate the interactions between the five major phytochemical constituents identified by GC–MS and the anti-apoptotic Bcl-2 protein (PDB ID: 2W3L). Since Bcl-2 plays a crucial role in suppressing the intrinsic apoptotic pathway in estrogen receptor-positive (ER-positive) breast cancer cells, inhibition of this protein represents an attractive strategy for anticancer drug discovery. Prior to docking, the docking protocol was validated by re-docking the co-crystallized native ligand into the Bcl-2 binding pocket. The resulting root mean square deviation (RMSD) value was 0.350 Å, which is substantially lower than the generally accepted threshold of 2.0 Å, confirming that the docking protocol was reliable for predicting ligand-binding conformations (Trott & Olson, 2010). The native ligand occupied the hydrophobic binding groove of Bcl-2 and interacted with key amino acid residues, including Phe63, Tyr67, Phe71, Met74, Val92, Leu96, and Ala108, which were subsequently used as reference residues for evaluating the binding modes of the investigated phytochemicals. The interaction profile was consistent with previous reports describing the active binding pocket of the Bcl-2 protein (Xu et al., 2019).

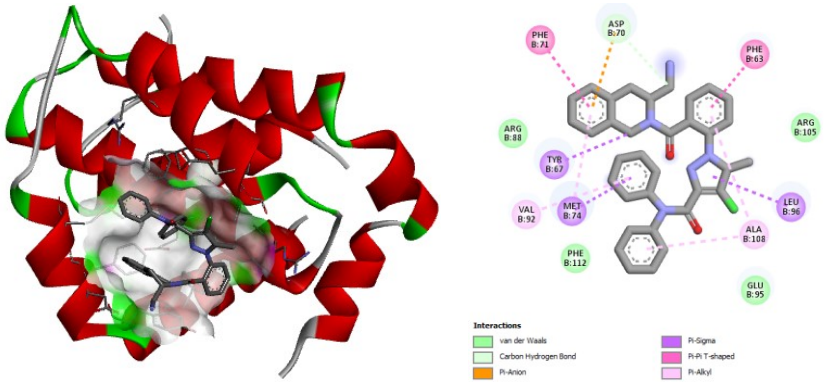


Figure 2. Two-dimensional (A) and three-dimensional (B) interaction profiles of the native ligand with the Bcl-2 protein.

Following docking validation, the five major phytochemicals identified from the chloroform extract of *P. zeylanica* were docked into the validated Bcl-2 binding site. The predicted binding free energy (ΔG), inhibition constant (Ki), and ligand–protein interactions are summarized in Table 4. Among the investigated compounds, stigmasterol exhibited the strongest predicted interaction with Bcl-2, with the lowest binding free energy (ΔG = −6.6 kcal/mol) and the smallest inhibition constant (Ki = 14.53 µM). Phytol, β-sitosterol, n-hexadecanoic acid, and cis-vaccenic acid displayed ΔG values ranging from −5.1 to −5.6 kcal/mol, indicating moderately favorable interactions with the receptor. Since more negative ΔG values correspond to more stable ligand–protein complexes and lower Ki values indicate stronger predicted inhibitory activity, these results suggest that stigmasterol possesses the highest affinity toward the Bcl-2 binding site among the identified phytochemicals (Du et al., 2016; Hidayah et al., 2024).

Table 4. Molecular docking results of the major phytochemicals identified from the chloroform extract of *P. zeylanica* against the Bcl-2 protein.

Molecules	ΔG (kcal/mol)	Ki (µM)	Interactions		
			Hydrogen	Hidrofobic	Van Der Waals
Bcl 2					
Stigmasterol	-6.6	14.53	Asp70	Tyr67, Met74, Leu96, Ala108	Phe63,Phe71, Val92, Glu111, Phe112, Glu73
Betasitosterol	-5.4	110.10		Met74, Val92,Leu96, Ala108, Arg105	Phe63, Asp70, Phe112, Glu95, Asn102
Phytol	-5.6	78.56	Ala108	Phe71, Tyr67, Phe63, Leu96, Val92, Phe112	Asp70, Met74, Glu111
Palmitic acid	-5.2	154.32		Phe71, Phe63, Tyr67, Leu96, Val92	Asp70, Met74, Ala108, Phe112
Cis Vaccenic acid	-5.1	182.67	Asp70	Phe71, Phe63, Tyr67, Met74, Leu96, Ala108, Val92	Phe112, Arg88, Glu95, Arg105

The superior binding affinity of stigmasterol can be attributed to its rigid steroidal skeleton and extensive hydrophobic surface, which facilitate stable accommodation within the hydrophobic binding groove of Bcl-2 (Ghosh et al., 2025). As illustrated in Figure 3, stigmasterol formed a conventional hydrogen bond with Asp70 together with multiple hydrophobic interactions involving Tyr67, Met74, Leu96, and Ala108, as well as several van der Waals interactions with Phe63, Phe71, Val92, Phe112, Glu73, and Glu111. Notably, several of these interacting residues overlap with those involved in native ligand binding, suggesting that stigmasterol occupies a similar binding region within the receptor. Such an interaction pattern supports the structural plausibility of stigmasterol binding to Bcl-2 and is consistent with previous reports describing steroidal compounds targeting apoptosis-related proteins (Dharmalingam et al., 2021).

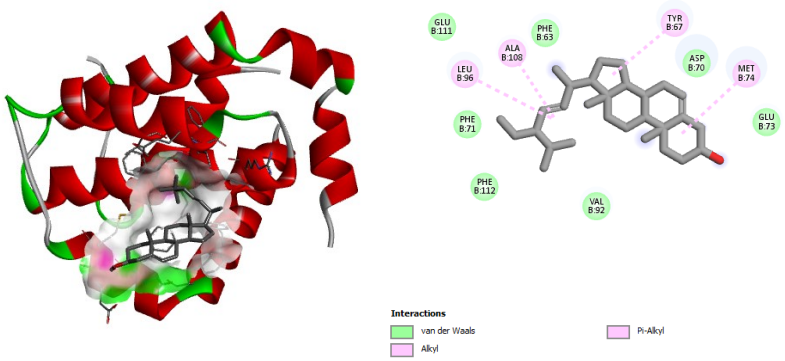


Figure 3. Two-dimensional (A) and three-dimensional (B) interaction profiles of stigmasterol with the Bcl-2 protein.

Although stigmasterol exhibited the strongest predicted interaction with Bcl-2, the remaining phytochemicals also interacted with several key residues within the same hydrophobic binding groove, including Phe63, Phe71, Tyr67, Met74, Val92, and Leu96. However, the rigid steroidal scaffold of stigmasterol likely provides greater structural complementarity to the receptor cavity than the more flexible diterpenoid and fatty acid structures, resulting in its superior binding affinity (Ghosh et al., 2025). The molecular docking results complement the in vitro cytotoxicity findings by providing a plausible molecular basis for the antiproliferative activity of the chloroform extract. Since Bcl-2 suppresses mitochondrial apoptosis, inhibition of this protein may promote caspase activation and apoptotic cell death (Adams & Cory, 2018; Delbridge et al., 2016). Among the identified compounds, stigmasterol is predicted to contribute most significantly because of its higher binding affinity, while the remaining phytochemicals may exert complementary effects. Nevertheless, molecular docking is only a predictive approach; therefore, further validation through apoptosis assays, Bcl-2 expression analysis, caspase activation assays, or molecular dynamics simulations is required (Trott & Olson, 2010).

CONCLUSIONS

The chloroform extract of *P. Zeylanica* leaves exhibited moderate cytotoxic activity against T47D breast cancer cells, with an IC_{50} value of 36.34 µg/mL. The extract contained appreciable levels of total phenolics and flavonoids, while GC–MS analysis identified 48 phytochemical compounds, with β-sitosterol, n-hexadecanoic acid, cis-vaccenic acid, phytol, and stigmasterol as the dominant constituents. Molecular docking against the Bcl-2 protein showed that stigmasterol exhibited the strongest binding affinity ($\Delta G = -6.6$ kcal/mol), suggesting its potential contribution to the cytotoxic activity of the extract. Overall, the integrated in vitro and in silico findings indicate that the chloroform extract of *P. zeylanica* is a promising source of natural anticancer compounds. Further studies involving compound isolation, selectivity evaluation, and in vivo validation are required to confirm its therapeutic potential.

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