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Camptothecin Anti-cancer Activity Against Breast Cancer Cells (MDA MB-231) Targeting the Gene Expression of Wnt/Beta-catenin Pathway - An In silico and In vitro Approach

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Abstract

Camptothecin, a potent anti-cancer agent, exhibits significant activity against MDA-MB-231 breast cancer cells by targeting the gene expression of the Wnt/β-catenin pathway. This pathway is crucial in cancer progression and cell proliferation. Camptothecin's effect on this pathway is elucidated through various assays and docking techniques. The DPPH assay demonstrates camptothecin's antioxidant potential, indicating its ability to neutralize free radicals. Additionally, nitric oxide assays reveal a significant enhancement in antioxidant properties, further supporting its therapeutic potential. Gene expression analysis provides insights into the molecular mechanisms underlying camptothecin's anti-cancer effects. The expression levels of key components of the Wnt/β-catenin pathway, including Wnt, β-catenin, APC, GSK3β, LP5, and Axin, are significantly altered in MDA-MB-231 cells upon camptothecin treatment. These changes suggest a disruption in the signaling pathway, which is vital for cancer cell survival and proliferation. The MTT assay results highlight camptothecin's capacity to inhibit cell growth in a time-dependent manner, underscoring its efficacy in reducing cancer cell viability over prolonged exposure. Moreover, docking studies indicate a high binding affinity between camptothecin and the Wnt/β-catenin pathway components, reinforcing the compound's role in modulating this critical signaling axis. Overall, camptothecin's multi-faceted approach, encompassing antioxidant activity and targeted gene expression modulation, presents a compelling case for its use in breast cancer therapy. The comprehensive analysis of its effects on the Wnt/β-catenin pathway offers valuable insights into its mechanism of action and potential as a therapeutic agent against aggressive breast cancer types like MDA-MB-231 cells.

Keywords: *Antioxidant Activity, Breast Cancer Therapy, Camptothecin, Gene Expression, MDA-MB-231, Novel Methods, Public Health, Wnt/β-catenin Pathway.*

Introduction

Breast cancer is one of the most prevalent malignancies affecting women worldwide, characterized by the uncontrolled growth of cells within the breast tissue. This disease can arise in different parts of the breast, including the ducts, lobules, or connective tissues, with ductal carcinoma and lobular carcinoma being the most common types [1]. The etiology of breast cancer is multifactorial, involving genetic, hormonal, and environmental factors. Key risk factors include age, family history of breast cancer, inherited mutations in genes such as BRCA1 and BRCA2, prolonged exposure to estrogen, obesity, and lifestyle factors such as alcohol consumption and physical inactivity.

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Breast cancer is classified into various subtypes based on hormone receptor status (estrogen receptor, progesterone receptor) and HER2 (human epidermal growth factor receptor 2) expression, which significantly influence prognosis and treatment strategies [2]. Detection and diagnosis of breast cancer typically involve a combination of physical examinations, mammography, ultrasound, and biopsy procedures to confirm the presence and type of cancer cells. Advanced diagnostic tools, such as MRI and molecular profiling, further aid in the characterization of the disease [3]. Treatment options for breast cancer are diverse and may include surgery, radiation therapy, chemotherapy, hormone therapy, targeted therapy, and immunotherapy. The choice of treatment is influenced by the cancer stage, subtype, patient's health status, and preferences. Despite advances in early detection and treatment, breast cancer remains a leading cause of cancer-related mortality among women. Ongoing research focuses on understanding the molecular mechanisms driving breast cancer, improving early detection methods, and developing more effective and personalized treatments [4]. Prevention strategies, such as lifestyle modifications, prophylactic surgeries for high-risk individuals, and chemoprevention, also play crucial roles in reducing the incidence and impact of breast cancer.

Phytocompounds, also known as phytochemicals, are naturally occurring chemical compounds found in plants. These bioactive substances are not essential nutrients but have significant health benefits and play crucial roles in plant growth, development, and defense against pathogens [5]. Phytocompounds can be classified into several categories, including alkaloids, flavonoids, tannins, saponins, phenolic acids, and terpenoids, each with unique properties and biological activities. For example, flavonoids, abundant in fruits, vegetables, and beverages like tea and wine, are renowned for their antioxidant, anti-inflammatory, and anticancer properties [6]. Similarly, alkaloids, found in plants such as coffee, tobacco, and poppy, exhibit a range of pharmacological effects, including pain relief, stimulation, and antimicrobial activity. The therapeutic potential of phytocompounds has garnered significant interest in the fields of medicine, nutrition, and pharmacology. These compounds contribute to the medicinal properties of many traditional herbs and have been incorporated into modern drug development. Phytocompounds are known to interact with various molecular targets in the human body, modulating signaling pathways and influencing gene expression [7]. For instance, curcumin from turmeric has been extensively studied for its anti-inflammatory and anticancer effects, while resveratrol from grapes is noted for its cardioprotective and longevity-promoting activities.

Camptothecin is a naturally occurring alkaloid derived from the Chinese tree Camptotheca acuminata and is renowned for its potent anticancer properties. Its mechanism of action primarily involves the inhibition of DNA topoisomerase I, an essential enzyme that relieves torsional strain during DNA replication and transcription by inducing transient single-strand breaks [8]. Camptothecin stabilizes the complex formed between DNA and topoisomerase I, preventing the re-ligation of the DNA strand and thereby causing DNA damage. This disruption leads to cell cycle arrest and apoptosis, particularly in rapidly dividing cancer cells, making camptothecin highly effective against various malignancies [9]. The clinical potential of camptothecin has led to the development of several derivatives, such as topotecan and irinotecan, which have been approved for cancer treatment. These derivatives are designed to improve the solubility, stability, and therapeutic index of camptothecin. Topotecan is commonly used in the treatment of ovarian cancer and small cell lung cancer, while irinotecan is effective against colorectal cancer [10]. Both drugs have

shown significant efficacy in clinical settings, often in combination with other chemotherapeutic agents to enhance their anticancer effects. Research has demonstrated that camptothecin and its derivatives can target multiple signaling pathways involved in cancer progression, including the Wnt/β-catenin pathway, which is crucial in regulating cell proliferation, differentiation, and survival [11]. By modulating the expression of genes associated with this pathway, camptothecin can inhibit tumor growth and metastasis. In this study, camptothecin exhibits significant anti-cancer activity against MDA-MB-231 breast cancer cells by targeting the Wnt/β-catenin pathway, altering key gene expressions, and demonstrating antioxidant properties. It effectively inhibits cell growth in a time-dependent manner and shows high binding affinity with pathway components, highlighting its therapeutic potential.

Materials and Methods

Chemicals and Reagents

Total RNA isolation reagents (TRIR) were received from Sigma Chemical Company, Missouri, USA. Krishgen Bio-systems. DPPH was procured from SRL chemicals. Gene specific primers were received from Eurofins Genomics, Bangalore, India. The phytocompound Squalene was procured from Sigma-Aldrich.

DPPH Activity

The assessment of DPPH radical scavenging activity was conducted to validate the camptothecin's anti-oxidant property. In this assay, 1.0 ml of a DPPH solution was mixed with 1.0 ml of the test extract at concentrations ranging from 0.1 to 0.5 mg/ml. The reaction mixture was then incubated for a specified period, allowing the DPPH radicals to interact with the antioxidants present in the extract. The decrease in absorbance of the solution, indicating the scavenging activity of the extract, was measured at a wavelength of 517 nm using a spectrophotometer. For comparison, ascorbic acid, a well-known antioxidant, was used as a standard at equivalent concentrations to those of the test extracts. This provided a benchmark to evaluate the effectiveness of the extracts in scavenging DPPH radicals [12].

Nitric Oxide Activity

The scavenging activity of the nitric oxide radical was to validate the camptothecin's anti-oxidant property. In this assay, 2 mL of 10 mM sodium nitroprusside in 0.5 mL of phosphate-buffered saline (pH 7.4) was combined with 0.5 mL of C. papaya extract at concentrations ranging from 100 to 500 µL and incubated at 25°C for 150 minutes. Following incubation, 0.5 mL of the reaction mixture was mixed with 1.0 mL of sulfanilic acid reagent. Subsequently, 1.0 mL of 0.1% (w/v) naphthylethylenediamine dihydrochloride was added, and the mixture was left to react for 30 minutes [13].

Cell Culture

The MDA-MB-231 cell line was acquired from the National Centre for Cell Science (NCCS) and cultured under standard conditions in a CO_2 incubator at 37°C. The culture medium used was DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. For the cytotoxicity assay, the cells were detached using 0.25% trypsin-EDTA and subsequently seeded into 96-well plates. This ensured that the cells were prepared in a manner suitable for evaluating their response to various treatments.

Cytotoxicity by MTT Assay

This study explored the cytocompatibility of camptothecin, a natural compound, against the MDA-MB-231 cell line using the MTT colorimetric assay. Cells were initially seeded at a density of $1x10^4$ cells per well in a 96-well plate and allowed to attach overnight. The compound, sterilized beforehand, was then diluted in a 1% dimethyl sulfoxide (DMSO)-DMEM media mixture to achieve concentrations ranging from 10 to 120 µM.

Next, the diluted samples were added to the wells and incubated for 24 to 72 hours in a CO_2 incubator. Following treatment, the supernatant was aspirated, and the wells were rinsed with 1X phosphate-buffered saline (PBS). MTT solution was subsequently introduced into each well and incubated for 1 hour. Formazan crystals formed were dissolved using DMSO, and the absorbance was measured at 590 nm to quantify cell viability. The percentage of viable cells was determined relative to a control, and the data were analyzed and graphed using GraphPad Prism software. Statistical significance was assessed using the paired Student's t-test to identify any meaningful differences between experimental conditions [14].

Gene Expression Analysis

For Real-Time PCR analysis, a meticulous reaction mixture was prepared using Takara SyBr green master mix. Specific forward and reverse primers were carefully designed for the target genes, as detailed in Table 1. The procedure included a melting analysis step, and a stable control was utilized for normalization. Results from this analysis were presented as fold changes relative to the control. Real-Time PCR was conducted using the CFX96 Touch Real-Time PCR machine from the USA, ensuring precise quantification of gene expression levels. This methodology enabled accurate assessment of the gene expression dynamics under investigation.

Table 1. RT-PCR Primer List

Gene	Forward Primer (5' -> 3')	Reverse Primer (5' -> 3')	Reference
Wnt	ATCCTCGAGCGTGTGATTTGA	GATGTGTTCAGGCACTCTCGA	[15]
β-catenin	TACTGTTTCTTGTTTCTTTGGGTT	AGAAGGACTTGAGGGCTCAG	[16]
APC	ATGAGAGGCTGAGATGGAAGG	CTTGGCAATGTGAGCTTGGT	[17]
GSK3β	CACTTGACCGAGAAGGTTGAG	AGCTGCGTAGCGTTGTAGA	[18]
LRP5	AGCCTGACAGCTGGAGAACT	TCGGACCAATGGATGGTGTT	[19]
Axin	TCTGCTGAGGCTGTATGAGG	TGTAGGCGTTGGAAGTGGTT	[20]
β-actin	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG	[21]

Molecular Docking

For the docking analysis, crystal structures of several proteins were obtained from the Protein Data Bank (PDB). These proteins include Wnt (PDB ID: 4F0A), β-catenin (PDB ID: 1JDH), GSK-3β (PDB ID: 1Q41), APC (PDB ID: 5A22), Axin (PDB ID: 1XTV). The structures were accessed via https://www.pdb.org/pdb. During the docking simulations, a grid box measuring 90 Å × 90 Å × 90 Å with a spacing of 0.45 Å was utilized. These specific parameters were chosen to ensure accurate calculations of drug molecule interactions with the target proteins. The results of the 3D structural docking analyses were visualized and analyzed using BIOVIA Discovery Studio software. This approach facilitated detailed examination of the binding interactions between the compounds and the protein targets.

Statistical Analysis

The study's statistical results were reported as mean values along with their respective standard deviations. Data analysis was performed using GraphPad Prism 8 software. For t-tests, significance levels of $*p<0.05$, $**p<$

0.01, and ***p< 0.001 were used to determine statistical significance.

Results

Anti-oxidant Activity of Camptothecin

The investigation revealed a comparable trend in the inhibition of anti-oxidant activity, with inhibition rates increasing proportionally with rising concentrations of both the camptothecin and the standard compound, ranging from 100 to 500 µg/ml. The maximum anti-oxidanteffect for both the extract and the standard compound was observed at the highest concentration of 500 µg/ml, as depicted in Figure 1 and detailed in Table 2. Moreover, the study's results demonstrated a dose-dependent increase in the percentage of inhibition against DPPH radicals for concentrations between 100 and 500 µg/ml for both the extract and the standard substance. The most significant antioxidant activity by nitric oxide was noted at concentrations of 400 and 500 µg/ml, as shown in Figure 2 and elaborated in Table 3. This pattern highlights the efficacy of both the extract and the standard compound in exhibiting antioxidant properties, particularly at higher concentrations, underscoring their potential therapeutic benefits.

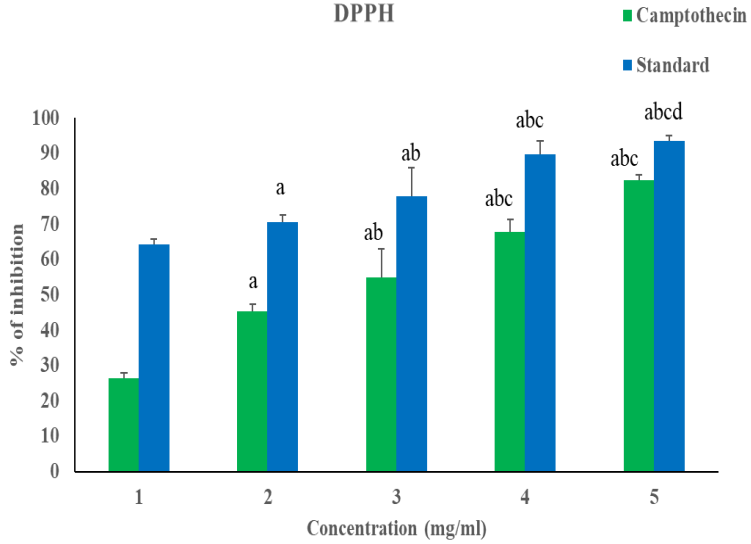

Figure 1. Represents the DPPH Activity (% of Inhibition). Values are Expressed in Mean ± SEM

Table 2. Represents the DPPH Activity (% of Inhibition). Values are Expressed in Mean ± SEM

Sample concentration (mg/ml)	% of inhibition for Camptothecin	% of inhibition for standard
100	26.38±1.5	62.69±1.0
200	45.2±2.1^{a}	69.57±0.4^{a}
300	54.92±7.4^{ab}	74.99±3.1^{ab}
400	67.65±3.7^{abc}	80.56±2.1^{abc}
500	82.27±1.6^{abcd}	85.42±0.54^{abcd}

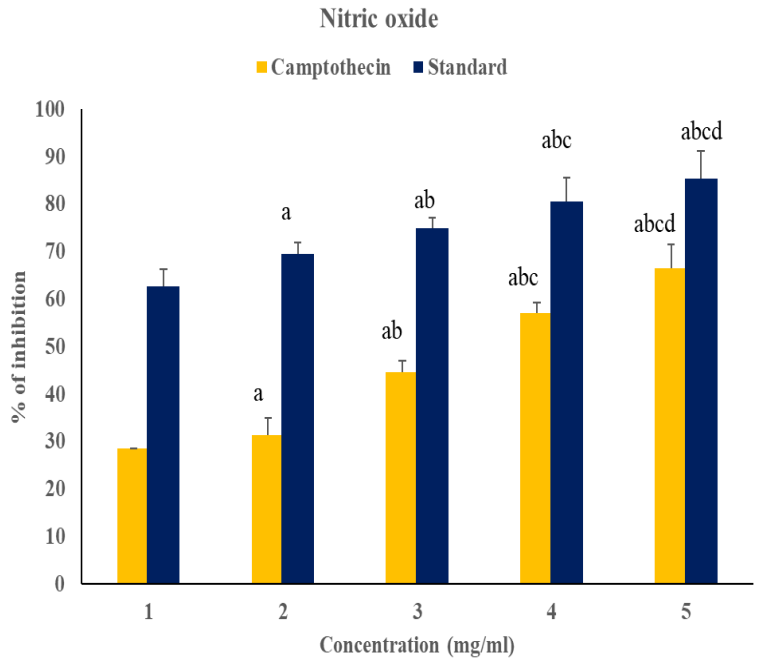

Figure 2. Represents the NO Activity (% of Inhibition). Values are Expressed in Mean ± SEM

Table 3. Represents the NO Activity (% of Inhibition). Values are Expressed in Mean ± SEM

Sample concentration (mg/ml)	% of inhibition for Camptothecin	% of inhibition for standard
100	28.54±3.7	64.13±3.7
20	31.27±2.4^{a}	70.51875±1.3^{a}
300	44.59±2.2^{ab}	77.94833±3.9^{ab}
400	57.12±5.1^{abc}	89.805±4^{abc}
500	66.55±5.8^{abcd}	93.46±0.8^{abcd}

Cytotoxicity Effects of Camptothecin

The cytotoxic effect of camptothecin on MDA MB 231 cells, in the context of breast cancer, was evaluated using the MTT assay. MDA MB 231 cells were exposed to increasing concentrations of camptothecin, ranging from 0 to 120 µM, over three different time intervals. The results, depicted in Figure 3, clearly demonstrated a significant, dose-dependent reduction in cell viability in response to camptothecin exposure over time. The cytotoxic effect of camptothecin was notably more pronounced after 48 hours of treatment compared to the 24and 72-hour interval, with even greater toxicity observed at 72 hours.

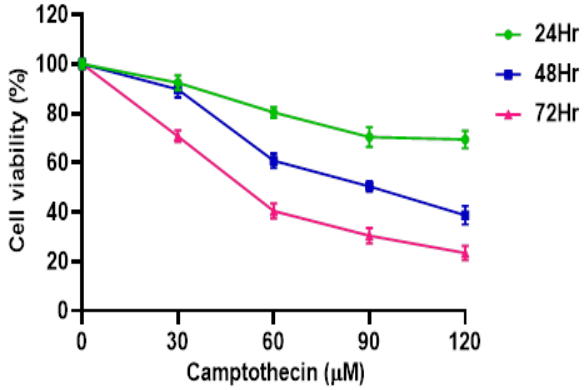

Figure 3. Cytotoxicity Assay of Cell Lines of MDA Cells on 24, 48, and 72 hr Time Intervals

Effects of Camptothecin the mRNA Expression Analysis of Wnt/β-catenin Signalling Molecules

Having established that camptothecin can inhibit cell growth in MDA MB 231 cells, our study aimed to explore the underlying molecular mechanisms in greater detail. Specifically, we investigated the molecular processes influenced by camptothecin in MDA MB 231 cells, focusing on its effects at 100 and 150µg doses over a 48-hour period. Real-time PCR analysis revealed significant findings: Camptothecin decreased the expression of Wnt, β-catenin, APC, GSK3β, and Axin, indicating its role in promoting metastasis. These results collectively suggest that camptothecin treatment in MDA MB-231 cells modulates the Wnt/GSK3β signaling pathway, providing insight into its therapeutic potential and molecular impact (Figure 4).

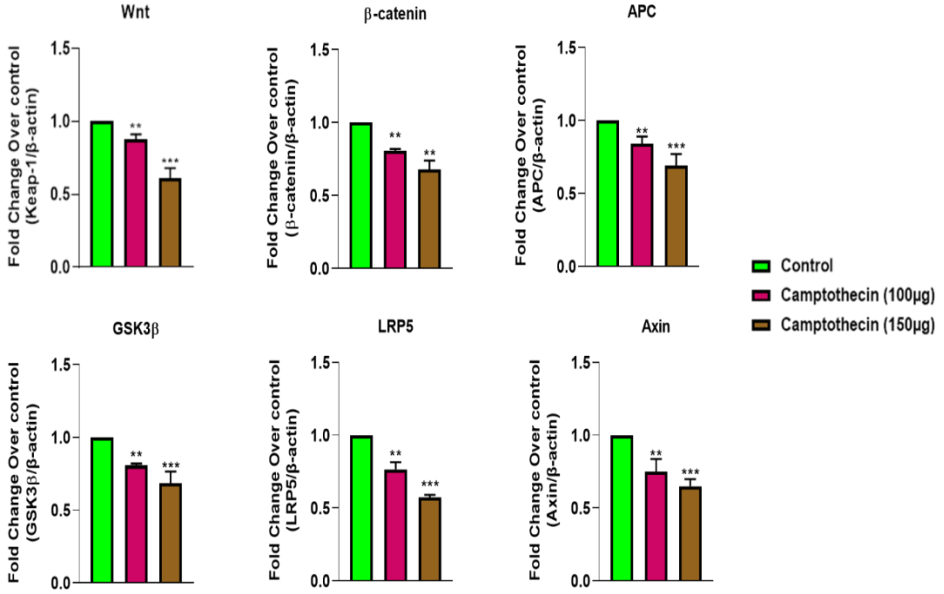

Figure 4. Effect of Camptothecin on Wnt, β-catenin, APC, GSK3β, LRP5, and Axin mRNA Expression

Molecular Interaction of Camptothecin with Wnt/ β-catenin Signalling Target Proteins

The molecular docking simulations provided valuable insights into the interactions between camptothecin and key proteins involved in metastasis. The binding energies, detailed in Table 4 and illustrated in Figure 5, reveal the strength and specificity of these interactions. Camptothecin exhibited notably high binding affinities with several critical proteins,

including Wnt (-7.4 kcal/mol), β-catenin (-7.6 kcal/mol), GSK3β (-8 kcal/mol), APC (-7.6 kcal/mol), and Axin (-7.3 kcal/mol). These findings suggest that camptothecin may significantly impact the Wnt signaling pathway, which is crucial in regulating cell proliferation, differentiation, and apoptosis. By binding effectively to these proteins, camptothecin could disrupt the signaling mechanisms that contribute to cancer cell survival and metastasis. The high binding energies observed indicate a strong interaction between camptothecin and the target proteins, highlighting its potential as an effective agent in cancer therapy. Specifically, its influence on the Wnt signaling pathway suggests that camptothecin could be a promising candidate for inducing apoptosis and inhibiting metastasis in breast cancer treatment. These molecular interactions underscore the therapeutic potential of camptothecin, providing a foundation for further research into its application in breast cancer therapy.

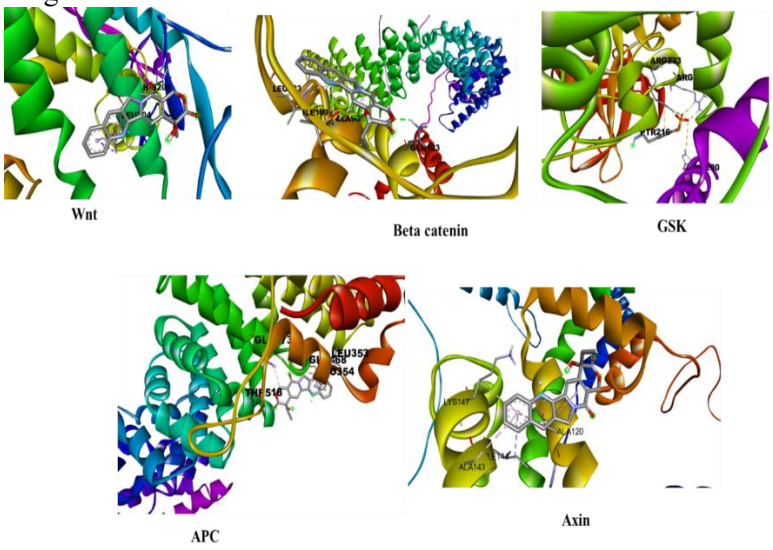

Figure 5. Molecular Docking Analysis of Selected Targets with Camptothecin

Table 4. Binding Affinity details of Selected Targets with Camptothecin

Targets	Binding affinity (Kcal/mol)
Wnt	-7.4
Beta-catenin	-7.6
GSk	-8
APC	-7.6
Axin	-7.3

Discussion

Breast cancer is one of the most common malignancies affecting women worldwide, characterized by the uncontrolled growth of cells in the breast tissue. This type of cancer can originate in various parts of the breast, including the ducts, lobules, or connective tissue, and has the potential to metastasize to other parts of the body, making it particularly dangerous [22]. Risk factors for breast cancer include genetic predisposition (such as BRCA1 and BRCA2 gene mutations), hormonal influences, lifestyle factors (such as diet, alcohol consumption, and lack of physical activity), and environmental exposures. Early

detection through screening methods like mammography and advancements in treatment strategies, including surgery, radiation therapy, chemotherapy, hormone therapy, and targeted biological therapies, have significantly improved survival rates. However, these treatments can have substantial side effects, prompting the exploration of alternative and complementary therapies [23, 24].

Phytotherapy, the use of plant-derived compounds for medicinal purposes, has emerged as a promising adjunct in breast cancer treatment. Various phytochemicals have shown potential anti-cancer properties, including the ability to inhibit cell proliferation, induce apoptosis, and prevent metastasis in various cancer including oral dieseas [25, 26]. For instance, compounds such as curcumin, resveratrol, and epigallocatechin gallate (EGCG) have been extensively studied for their anti-inflammatory, antioxidant, and anti-tumor effects. Boswellic acid, derived from the resin of the Boswellia tree, has demonstrated efficacy in inducing cell cycle arrest and promoting apoptosis in cancer cells, including those in breast cancer. Camptothecin, an alkaloid derived from the *Camptotheca acuminata* tree, has shown significant interactions with key proteins involved in cancer progression, suggesting its potential in targeted cancer therapy [27]. The integration of phytotherapy into conventional breast cancer treatment regimens offers a holistic approach, potentially enhancing therapeutic outcomes while minimizing adverse effects [28, 29]. Ongoing research continues to explore the mechanisms of action, efficacy, and safety of various phytochemicals, aiming to establish their role in comprehensive cancer care.

The investigation revealed a consistent pattern in the inhibition of antioxidant activity, with inhibition rates increasing proportionally to the rising concentrations of both camptothecin and the standard compound, spanning from 100 to 500 µg/ml [30]. The peak antioxidant effect for both the extract and the standard compound was observed at the highest concentration of 500 µg/ml, as shown in Figure 1 and detailed in Table 2. Furthermore, the study demonstrated a dose-dependent increase in the percentage of inhibition against DPPH radicals at concentrations between 100 and 500 µg/ml for both the extract and the standard substance [31]. The most significant antioxidant activity, measured by nitrous oxide, was noted at concentrations of 400 and 500 µg/ml, as presented in Figure 3 and elaborated in Table 3. This trend underscores the efficacy of both the extract and the standard compound in exhibiting antioxidant properties, particularly at higher concentrations, highlighting their potential therapeutic benefits.

To evaluate the cytotoxic effect of camptothecin on MDA-MB-231 cells within the context of breast cancer, the MTT assay was employed. MDA-MB-231 cells were exposed to increasing concentrations of camptothecin, ranging from 0 to 120 µM, over three different time intervals [32]. The results, depicted in Figure 4, clearly demonstrated a significant, dose-dependent reduction in cell viability in response to camptothecin exposure over time. The cytotoxic effect of camptothecin was notably more pronounced after 48 hours of treatment compared to the 24-hour interval, with even greater toxicity observed at 72 hours. Having established that camptothecin can inhibit cell growth in MDA-MB-231 cells, the study aimed to explore the underlying molecular mechanisms in greater detail. Specifically, the investigation focused on the molecular processes influenced by camptothecin in MDA-MB-231 cells, examining its effects at 100 and 150 µg doses over a 48-hour period. Real-time PCR analysis revealed significant findings: camptothecin decreased the expression of Wnt, β-catenin, APC, GSK3β, and Axin, indicating its role in modulating pathways associated with metastasis. These results collectively suggest that camptothecin treatment in MDA-MB-231 cells impacts the Wnt/GSK3β signaling

pathway, providing insights into its therapeutic potential and molecular impact.

Molecular docking simulations offered valuable insights into the interactions between camptothecin and key proteins involved in metastasis. The binding energies, detailed in Table 4 and illustrated in Figure 5, reveal the strength and specificity of these interactions (Chen et al., 2023). Camptothecin exhibited notably high binding affinities with several critical proteins, including Wnt (-7.4 kcal/mol), β-catenin (-7.6 kcal/mol), GSK3β (-8 kcal/mol), APC (-7.6 kcal/mol), and Axin (-7.3 kcal/mol). These findings suggest that camptothecin may significantly influence the Wnt signaling pathway, which is crucial in regulating cell proliferation, differentiation, and apoptosis. By effectively binding to these proteins, camptothecin could disrupt the signaling mechanisms that contribute to cancer cell survival and metastasis. The high binding energies observed indicate a strong interaction between camptothecin and the target proteins, highlighting its potential as an effective agent in cancer therapy. Specifically, its influence on the Wnt signaling pathway suggests that camptothecin could be a promising candidate for inducing apoptosis and inhibiting metastasis in breast cancer treatment [33]. These molecular interactions underscore the therapeutic potential of camptothecin, providing a solid foundation for further research into its application in breast cancer therapy.

Overall, this comprehensive investigation highlights the multifaceted potential of camptothecin in breast cancer treatment. Its significant antioxidant properties, coupled with its strong cytotoxic effects on cancer cells and its ability to modulate crucial signaling pathways, position camptothecin as a promising therapeutic agent. Future research should continue to explore these interactions and their implications, with the goal of developing more effective and targeted treatments for breast cancer.

Conclusion

Camptothecin demonstrates significant anti-cancer activity against MDA-MB-231 breast cancer cells by effectively targeting the Wnt/β-catenin signaling pathway. This pathway is crucial for cancer progression and cell proliferation. Through DPPH and nitric oxide assays, camptothecin's antioxidant properties are highlighted, suggesting its ability to neutralize free radicals. Gene expression analysis reveals significant alterations in key components of the Wnt/β-catenin pathway, indicating disruption of this vital signaling axis. The MTT assay further underscores camptothecin's efficacy in inhibiting cell growth in a time-dependent manner. Molecular docking studies show a high binding affinity between camptothecin and Wnt/β-catenin pathway components, reinforcing its role in modulating this critical pathway. Overall, camptothecin's combined antioxidant activity and targeted gene expression modulation present a compelling case for its therapeutic potential in treating aggressive breast cancer types, providing valuable insights into its mechanism of action and reinforcing its promise as a cancer therapy.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

Acknowledgement

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