RESEARCH PAPER

Unveiling Therapeutic Avenues: In Silico Analysis Of Breast Cancer Proteins And Ligands For Drug Discovery

Soorya Ganesh[1], Jumaana Hazeen Naina Mohamed[2], Eswaran Maheswaran[1], Dhanraj Ganapathy[1,*]

[1]Department Of Prosthodontics, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.
[2]Saveetha Medical College, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.

***Corresponding Author:**
Dhanraj Ganapathy, Department Of Prosthodontics, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India.
Email: dhanraj@saveetha.com

Abstract
In Silico Studies Were Conducted To Investigate The Potential Of Thymol, A Natural Compound Found In Herbs Like Thyme And Oregano, For Breast Cancer Treatment. The Study Analyzed How Thymol Interacts With Key Molecular Targets (Brca-2, Galectin-1, And Mmp-2) Linked To Breast Cancer. The Analysis Predicted Strong Binding Affinity Between Thymol And Brca-2 (Binding Efficiency: -6.8) Compared To Galectin-1 (-5.6) And Mmp-2 (-5.7), Suggesting That Thymol'S Interaction With Brca-2 Might Be Particularly Favorable For Anti-Cancer Activity. Furthermore, In Silico Analysis, Swissadme Predicted High Water Solubility And Gastrointestinal Absorption For Thymol, Indicating Good Drug-Like Properties. In Conclusion, These In Silico Findings Support The Potential Of Thymol-Based Therapeutics For Breast Cancer Treatment Due To Favorable Binding Interactions With Key Targets And Promising Drug Characteristics. This Paves The Way For Further Research And Development Of Thymol-Based Therapies For Breast Cancer.

Keywords: Breast Cancer, Brca-2, Galectin-1, Mmp-2, Thymol.
How To Cite This Article: Ganesh S, Mohamed JHN, Maheswaran E, Ganapathy D. Unveiling therapeutic avenues: in silico analysis of breast cancer proteins and ligands for drug discovery. Int J Drug Deliv Technol. 2026;16(9s): 463-467; Doi: 10.25258/Ijddt.16.9s.46

1. Introduction

Breast cancer (BC) is the most prevalent cancer type, affecting women worldwide. BC arises from the breast tissue such as lobules and the ducts, often forming lumps or masses (Feng et al. 2018). In rare cases, men can also develop breast cancer (1%) and it causes 0.2% of deaths in men (Gucalp et al. 2019). This disease casts a long shadow, with an estimated 2.3 million new cases diagnosed globally (Łukasiewicz et al. 2021). Despite these challenges, significant advancements have been made in breast cancer diagnostic and therapeutic approaches. For example, early diagnostic and therapeutic approaches can control or mitigate the human mortality rate (Ginsburg et al. 2020).

Innovative drug design and drug delivery strategies are vital in cancer treatment, enabling the development of targeted therapies that minimize systemic toxicities and maximize therapeutic efficacy, ultimately improving patient quality of life (Mansouri et al. 2024). Hence, modern cancer drug development emphasizes targeting specific molecular pathways implicated in tumor progression, often utilizing nano-based delivery systems to enhance immunotherapeutic efficacy (Han et al. 2024). Tamoxifen, for example, a selective estrogen receptor modulator, is used to antagonize estrogen receptor signalling, thereby inhibiting the proliferative effects of estrogen in estrogen receptor-positive breast cancer (Patel et al. 2018).

Cancer cells enabling the development of drug resistance through various mechanisms such as DNA repair mechanism, drug efflux and apoptosis suppression, which limiting therapeutic efficacy. (Mansoori et al. 2017). Furthermore, current cancer drug development faces challenges related to target

specificity and systemic toxicities. Some conventional therapies can induce unwanted side effects such as fatigue, nausea and vomiting (Gupta et al. 2021). Prolonged drug research, drug development and drug delivery system have been contributed to the elevated cost of new pharmaceuticals, thereby restricting patient access, especially in low-income countries (Vincent Rajkumar 2020; Jalali et al. 2022). To overcome these limitations, researchers are continuously exploring new avenues, such as personalized medicine and combination therapies.

Thymol, a natural compound found in thyme, oregano, and other herbs, possesses various biological activities with promising applications in breast cancer treatment (Vahitha et al. 2024). Previous research suggests thymol exerts its anticancer effects through multiple mechanisms, including inducing cell death (apoptosis) in breast cancer cells, hindering their proliferation, and suppressing their ability to invade and migrate (Elbe et al. 2020). To further understand thymol interaction with breast cancer, this study employed *in silico* analysis, a computational approach. The analysis focused on how thymol interacts with key molecular targets associated with breast cancer, such as BRCA genes, Human Galectin-2, and matrix metalloproteinase-2 (MMP-2). By identifying and characterizing these protein-ligand interactions, the study aimed to pave the way for the development of more effective breast cancer therapies using thymol.

In silico studies offer a powerful tool to investigate the potential of natural compounds like thymol for breast cancer treatment. This approach utilizes computational methods to explore interactions between thymol and key molecular targets associated with the disease. Recently, *in silico* studies focused on how thymol interacts with BRCA-2, Galectin-1, and MMP-2, all playing crucial roles in breast cancer development. The analysis predicted a strong binding affinity between thymol and BRCA-2, suggesting a potentially significant role for this interaction in thymol's anti-cancer effects. Overall, *in silico* studies provide valuable insights into thymol potential for breast cancer therapy.

Materials and Methods

Study of Breast Cancer related Proteins

Breast cancer research involves a detailed study of proteins and ligands that play crucial roles in the disease pathogenesis. Novel proteins such as Human Galectin-2, MMP-2, and BRCA2 have been considered as a perfect protein for the study of breast cancer. Understanding the structural and functional aspects of these proteins aids in identifying their role in tumor growth and metastasis. Ligand binding to these proteins can modulate their activity, making ligand studies vital for therapeutic advancements.

Selection of Proteins and Ligands

In protein-ligand studies, selecting proteins such as BRCA2, Human Galectin-2, and MMP-2 is driven by their critical roles in cancer biology and therapeutic potential. BRCA2 is pivotal in DNA repair mechanisms; mutations in BRCA2 increase breast cancer risk, making it a key target for studying interactions with potential therapeutic ligands. Human Galectin-2 is involved in cell-cell and cell-matrix interactions, playing a role in cancer progression and metastasis. Studying ligands that modulate Galectin-2 activity can reveal mechanisms to inhibit tumor spread. MMP-2 facilitates cancer invasion and metastasis. MMP-2 and ligand interactions can potentially prevent tumor dissemination. Therefore, we are investigating the binding interactions between thymol and proteins (BRCA2, Human Galectin-2, and MMP-2) can be employed to study protein-ligand interaction and its potential as a therapeutic agent.

Molecular Docking and Simulation of Proteins using CB-Dock2

CB-Dock2, an enhanced version of the CB-Dock tool, facilitates this process by providing accurate docking predictions with user-friendly features as previously described (Liu et al. 2022). The following steps outline the process of conducting molecular docking using CB-Dock2. Obtain the 3D structures of target proteins (BRCA2, Human Galectin-2, and MMP-2) from databases like the Protein Data Bank (PDB). Prepare the ligand structure (thymol) using molecular modelling software and save it in PDB format. Access the CB-Dock2 web interface and upload the protein and ligand protein data bank files. CB-Dock2 automatically predicts potential binding sites on the protein surface using its cavity detection algorithm. Finally, we review and select the predicted binding pockets for docking.

CB-Dock2 uses a fast-docking algorithm to generate multiple binding poses. Particularly, the docking data includes binding scores and binding poses. To examine the binding interactions between thymol and the target proteins (BRCA2, Human Galectin-2, and MMP-2), all the top-ranked poses

were visualized and analysed. CB-Dock2 integrated visualization tools have been utilized to analyse hydrogen bonds, and hydrophobic interactions.

Evaluates the pharmacokinetics, and medicinal chemistry of Thymol using SwissADME

SwissADME web tool was used to study valuable insights of thymol and its absorption, distribution, metabolism, and excretion profiles. Initially, the chemical structure of thymol was entered into the SwissADME tool, which can be input as a SMILES string or drawn using the interface. We can generate the molecular structure and essential physicochemical properties using SwissADME.

Results and Discussion

Molecular docking is essential to predict the stronger binder, ligand-receptor structure and screen a database of the target compounds (Meng et al. 2011). In this study, CB-Dock2 facilitates the molecular docking process, making it accessible and efficient for studying protein-ligand interactions, such as those involving thymol and breast cancer-related proteins including BRCA-2, Galectin-1 and MMP-2. SwissADME predicts high gastrointestinal absorption for thymol, indicating best oral bioavailability. The tool provides several pharmacokinetics properties such as the blood-brain barrier (BBB) permeability and P-glycoprotein substrate status (Rai et al. 2023). Thymol has the potential to cross the BBB, suggesting potential central nervous system activity.

In silico data predicted the binding affinity and mode of interaction between thymol and molecular targets (BRCA-2, Galectin-1 & MMP-2), providing valuable insights into the potential mechanisms underlying thymol's anticancer effects. The compounds thymol displayed binding energy including -5.6, -4.7, -6.8, -4.9, -4.8, against BRCA2 (1mje) at the site of C1-C5, respectively. Similarly, Human Galectin-1 showed -5.5, -4.2, -5.6, -4.5, -4.3 the CurPocket value and MMP-2 showed -5.7, -4.8, -4.3, -4.8, -4.1 the CurPocket value against thymol ligands at the site of C1-C5, respectively. Overall, thymol had the highest binding value with BRCA2 compared to the other proteins like Human Galectin-1 and MMP-2.

Thymol had maximum binding energy (-6.5) with BRCA2 (3EU7) (Hossain et al. 2022). The results suggest that BRCA-2 – thymol binding may enhance anti-cancer activity. BRAC-2 (PDB:1mje) has high binding efficiency (-6.8) with thymol, compared with Galectin-1 (-5.6) & MMP-2 (-5.7). Thymol has high water solubility and also has high gastrointestinal (GI) absorption capacity when analyzed using SwissADME. As a result, *in silico* studies contribute to the rational design and optimization of thymol-based therapeutic agents for breast cancer treatment.

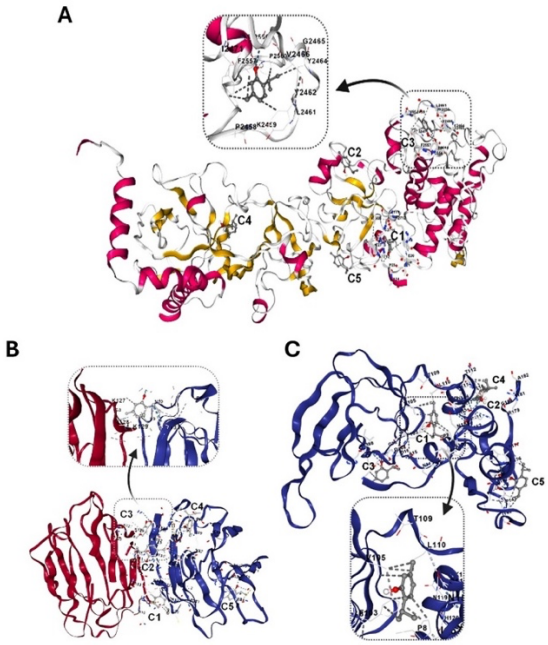

Figure 1. *In silico* analysis of proteins and ligand interactions. **A.** Interactions and docked 3D structures of thymol (Ligand) with Breast Cancer 2 – BRCA2 (1mje). Box indicated the highest interaction between protein and ligand with the vinascore (-6.8). **B.** Interactions and docked 3D structures of thymol (Ligand) with Human Galectin 1 (3oy8). Box indicated the highest interaction between protein and ligand with the vinascore (-5.6). **C.** Interactions and docked 3D structures of thymol (Ligand) with Matrix metalloproteinase-2 (MMP-2) - (1br9). Box indicated the highest interaction between protein and ligand with the vinascore (-5.7).

BRCA2-thymol interaction has strong binding interactions compared to Human Galectin 2 – Thymol and MMP 2 – Thymol interaction. The discussion comparing the binding interactions of BRCA2-thymol, Human Galectin 2-thymol, and MMP 2-thymol is quite intriguing. Each interaction involves a different protein and thymol, a natural compound known for its potential therapeutic properties. BRCA2 interaction with thymol could suggest implications in cancer therapy, potentially enhancing the gene function in repairing damaged DNA.

Table 1. Proteins (BACA-2, Human Galectin-1, and MMP-2) and Ligand (Thymol) interaction with the CurPocket value.

Protein/Ligand	C1	C2	C3	C4	C5
BRCA-2	-5.6	-4.7	-6.8	-4.9	-4.8
Human Galectin-1	-5.5	-4.2	-5.6	-4.5	-4.3
MMP-2	-5.7	-4.8	-4.3	-4.8	-4.1

On the other hand, Human Galectin 2 is a protein involved in various cellular processes, including inflammation and cancer progression (Lima et al. 2023). Its interaction with thymol might have implications in modulating these processes, potentially offering therapeutic avenues in inflammatory conditions or cancer treatment. Meanwhile, MMP-2 plays a role in tissue remodeling and is often associated with cancer progression and metastasis (Mustafa et al. 2022). Its interaction with thymol could hint at potential therapeutic strategies targeting MMP 2 activity, which could be valuable in inhibiting cancer spread. Comparing the strength of binding interactions among these interactions could shed light on which pathway might be more promising for therapeutic interventions. If the BRCA2-thymol interaction indeed exhibits stronger binding, it could indicate a potentially more significant impact on cancer therapy through DNA repair mechanisms (Dashtaki et al. 2020; Hossain et al. 2022). However, further experimental validation and detailed molecular studies would be necessary to confirm and understand these observations better.

Thymol has a logP value indicating moderate lipophilicity, which balances solubility and membrane permeability. SwissADME evaluates thymol against established rules like Lipinski's rule, Veber's rule, confirming its drug-like characteristics (Bitew et al. 2021; Satish et al. 2023). Synthetic accessibility score of thymol suggests it is relatively easy to synthesize, making it a feasible candidate for drug development. Thymol properties highlight its compliance with optimal ranges for bioavailability, indicating a favourable pharmacokinetic profile. Using SwissADME, we can efficiently predict the pharmacokinetic behaviour and medicinal chemistry properties of thymol, supporting its potential as a therapeutic agent in cancer treatment (Varshan and Sankar 2024).

Conclusions

This study utilized computer modeling (*in silico*) to explore the potential of thymol, a natural compound from herbs like thyme and oregano, for treating breast cancer. The analysis focused on how thymol interacts with key molecules (BRCA-2, Galectin-1, and MMP-2) are involved in breast cancer development. The results predicted a strong binding affinity between thymol and BRCA-2, suggesting a potentially favorable anti-cancer mechanism. Additionally, the *in-silico* analysis indicated good drug-like properties for thymol, including high water solubility and gastrointestinal absorption. These findings support the potential of thymol-based therapeutics for breast cancer. Further research and development are guaranteed to explore this promising avenue.

References

Bitew M, Desalegn T, Demissie TB, Belayneh A, Endale M, Eswaramoorthy R (2021) Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. PLoS One 16:e0260853 doi: 10.1371/journal.pone.0260853

Dashtaki A, Mahjoub S, Zabihi E, Pourbagher R (2020) The Effects of Pre-Treatment and Post-Treatment of Thymol against tert-Butyl Hydroperoxide (t-BHP) Cytotoxicity in MCF-7 Cell Line and Fibroblast Derived Foreskin. Rep Biochem Mol Biol 9:338-347 doi: 10.29252/rbmb.9.3.338

Elbe H, Yigitturk G, Cavusoglu T, Uyanikgil Y, Ozturk F (2020) Apoptotic effects of thymol, a novel monoterpene phenol, on different types of cancer. Bratisl Lek Listy 121:122-128 doi: 10.4149/bll_2020_016

Feng Y et al. (2018) Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis 5:77-106 doi: 10.1016/j.gendis.2018.05.001

Ginsburg O et al. (2020) Breast cancer early detection: A phased approach to implementation. Cancer 126 Suppl 10:2379-2393 doi: 10.1002/cncr.32887

Gucalp A et al. (2019) Male breast cancer: a disease distinct from female breast cancer. Breast Cancer Res Treat 173:37-48 doi: 10.1007/s10549-018-4921-9

Gupta K, Walton R, Kataria SP (2021) Chemotherapy-Induced Nausea and Vomiting: Pathogenesis, Recommendations, and New Trends. Cancer Treatment and Research Communications 26:100278 doi: https://doi.org/10.1016/j.ctarc.2020.100278

Han MM, Fan YK, Zhang Y, Dong ZQ (2024) Advances in herbal polysaccharides-based nano-drug delivery systems for cancer immunotherapy. J Drug Target 32:311-324 doi: 10.1080/1061186x.2024.2309661

Hossain R et al. (2022) Natural Compounds or Their Derivatives against Breast Cancer: A Computational Study. Biomed Res Int 2022:5886269 doi: 10.1155/2022/5886269

Jalali R, Nogueira-Rodrigues A, Das A, Sirohi B, Panda PK (2022) Drug Development in Low- and Middle-Income Countries: Opportunity or Exploitation? American Society of Clinical Oncology Educational Book:3-10 doi: 10.1200/edbk_10033

Lima T et al. (2023) Galectin-3 in prostate cancer and heart diseases: a biomarker for these two frightening pathologies? Mol Biol Rep 50:2763-2778 doi: 10.1007/s11033-022-08207-1

Liu Y, Yang X, Gan J, Chen S, Xiao Z-X, Cao Y (2022) CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic Acids Research 50:W159-W164 doi: 10.1093/nar/gkac394

Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A (2021) Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers (Basel) 13 doi: 10.3390/cancers13174287

Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B (2017) The Different Mechanisms of Cancer Drug Resistance: A Brief Review. Adv Pharm Bull 7:339-348 doi: 10.15171/apb.2017.041

Mansouri S, Alharbi Y, Alqahtani A (2024) Current status and prospects for improved targeted delivery approaches for cancer. Pathology - Research and Practice 253:154993 doi: https://doi.org/10.1016/j.prp.2023.154993

Meng XY, Zhang HX, Mezei M, Cui M (2011) Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 7:146-157 doi: 10.2174/157340911795677602

Mustafa S, Koran S, AlOmair L (2022) Insights Into the Role of Matrix Metalloproteinases in Cancer and its Various Therapeutic Aspects: A Review. Front Mol Biosci 9:896099 doi: 10.3389/fmolb.2022.896099

Patel S, Homaei A, Raju AB, Meher BR (2018) Estrogen: The necessary evil for human health, and ways to tame it. Biomed Pharmacother 102:403-411 doi: 10.1016/j.biopha.2018.03.078

Rai M et al. (2023) Herbal concoction Unveiled: A computational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMs). Current Research in Toxicology 5:100118 doi: https://doi.org/10.1016/j.crtox.2023.100118

Satish S, Rengasamy G, Sekaran S, Sankaran K, Veeraraghavan VP, Eswaramoorthy R (2023) Molecular docking analysis of protein filamin-A with thioazo compounds. Bioinformation 19:99-104 doi: 10.6026/97320630019099

Vahitha V, Lali G, Prasad S, Karuppiah P, Karunakaran G, AlSalhi MS (2024) Unveiling the therapeutic potential of thymol from Nigella sativa L. seed: selective anticancer action against human breast cancer cells (MCF-7) through down-regulation of Cyclin D1 and proliferative cell nuclear antigen (PCNA) expressions. Mol Biol Rep 51:61 doi: 10.1007/s11033-023-09032-w

Varshan I, Sankar S (2024) Molecular Docking Analysis of Hydroxyclavicol and Eugenol From Betel Leaves Against Outer Membrane Protein (OmpH) of Dialister pneumosintes. Cureus 16:e53809 doi: 10.7759/cureus.53809

Vincent Rajkumar S (2020) The high cost of prescription drugs: causes and solutions. Blood Cancer J 10:71 doi: 10.1038/s41408-020-0338-x