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An in silico comparative docking analysis of breast cancer drugs and natural compounds targeting DLAT and ATOX1 proteins

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Abstract

Introduction: Breast cancer is common among women, influenced by genetic and environmental factors. Proteins DLAT and ATOX1 contribute to disease. DLAT is part of the pyruvate dehydrogenase complex, involved in metabolism, while ATOX1 regulates copper. Chemotherapy drugs like Epirubicin, Xeloda, and Gemcitabine prevent cancer growth. Natural compounds such as Formononetin and Curcumin also show anticancer potential. Formononetin induces apoptosis and inhibits invasion, while Curcumin has antioxidant and anti-inflammatory effects against cancer.

Materials and methods: In this study, we used molecular docking to explore how these drugs and natural compounds interact with the DLAT and ATOX1 proteins. We obtained the protein structures from the PDB database and the drug structures from PubChem, and docking was performed using Pyrx software version 0.8 to evaluate binding affinities.

Results: The results showed that all the drugs and plant compounds had a good ability to bind to both proteins, but the binding to DLAT was stronger. Among the drugs, Xeloda performed the best with a binding affinity of -7.5 kcal/mol, and among the plant compounds, Formononetin showed the highest effectiveness with a binding affinity of -8.3 kcal/mol.

Conclusion: Natural compounds such as formononetin and curcumin may offer insights into potential interactions with proteins involved in breast cancer, but further studies are needed to confirm their effectiveness.

Keywords: Breast cancer, DLAT, ATOX1, Molecular docking

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Introduction

Breast Cancer and Molecular Factors in Tumor Progression

Breast cancer is one of the most significant health challenges for women and most frequently diagnosed cancer among women (1). Most breast tumors begin in the mammary ducts, and under prolonged exposure to carcinogenic factors, they may evolve into benign growths or even invasive and metastatic carcinomas (2).

Role of DLAT in Mitochondrial Metabolism and Tumor Biology

Dihydrolipoamide S-acetyltransferase (DLAT) is a mitochondrial protein that serves as the E2 component of the pyruvate dehydrogenase complex. It is located in the inner mitochondrial membrane and participates in glucose metabolism by converting pyruvate-derived acetyl groups into coenzyme A (3). Recently, Tsotkov et al. introduced the concept of "coproptosis," a copper-induced cell death mechanism linked to mitochondrial respiration and the lipoic acid pathway (4). Upon oligomerization, DLAT becomes susceptible to copper-induced toxic stress and leads to cell death, highlighting its importance in the process of copper toxicity and its potential role in cancer therapy (5). Recent studies have shown that DLAT expression is elevated in patients who are resistant to anti-PD-L1/PD-1 therapies, suggesting that DLAT may also serve as a predictive marker for breast cancer (BRCA) resistance to immunotherapy (6).

Role of ATOX1 in Copper Homeostasis and Cancer Progression

Antioxidant protein 1 (ATOX1) is a copper metallochaperone that is upregulated in several cancers, including breast, colorectal, uterine, and liver tumors (Figure 1.B), but downregulated in cancers such as cholangiocarcinoma and pancreatic tumors (7).ATOX1 maintains copper balance in cells by delivering copper to ATP7A and ATP7B in the trans Golgi network, which promotes the activation of copper dependent enzymes like ceruloplasmin and lysyl oxidase (8). Interestingly, nuclear ATOX1 can also function as a transcription factor, contributing to processes such as activin-induced migration and colony formation in cancer cells (9). ATOX1 is significantly upregulated in breast cancer cells. Increased ATOX1 expression is associated with poor prognosis in individuals with early-stage breast cancer (10). DCAC50, a small molecule inhibitor of ATOX1 and CCS, reduces cell proliferation, inhibits angiogenesis, increases copper levels, and increases oxidative stress, thereby inducing apoptosis in TNBC cells and enhancing the cytotoxicity of paclitaxel (11).

Coproptosis may also be a novel pathway to combat cancer cells. Therefore, disruption of ATOX1-mediated processes could be beneficial for the efficacy of anticancer therapies, although this should be considered with caution due to the dual role of copper in cancer (12).

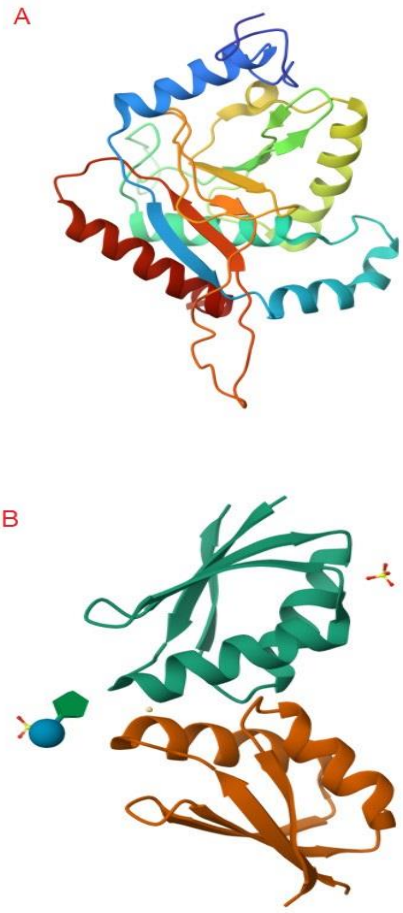

Figure 1. Three-dimensional structure of the proteins DLAT and Atox1. a: DLAT protein. b: Atox1 protein.

Epirubicin

Epirubicin is an anthracycline drug and the 4′-epimer of doxorubicin (Figure 2.A). It has been in clinical use in the United States since 1999 and is now marketed in more than 80 countries for the treatment of breast cancer as well as other malignancies. The anticancer activity of epirubicin involves several mechanisms: intercalation into DNA, inhibition of topoisomerase II, generation of reactive oxygen species, and subsequent disruption of DNA, RNA, and protein synthesis (13). These same mechanisms are also linked to the cardiotoxic side effects observed with doxorubicin and other anthracyclines.

Xeloda

Xeloda is an oral fluoropyrimidine carbamate (Figure 2.B) and is currently the only approved treatment for patients whose disease has progressed following anthracycline and taxane-based therapy. It is designed to be selectively activated within tumor tissue, where it generates therapeutically active concentrations of 5-fluorouracil (5-FU) (14-16). Because thymidine phosphorylase the enzyme responsible for the final activation step of Xeloda is more active in tumors than in normal tissues, intratumoral levels of 5-FU are higher than those in plasma. This tumor-selective activation, along with reduced systemic exposure, improves the therapeutic index of Xeloda (17,18).

Gemcitabine

Gemcitabine is one of the most active agents used against breast cancer and has received full approval for its clinical application (Figure 2.C). It is a deoxycytidine analog with potent anticancer activity and a favorable therapeutic index (19). Once administered, gemcitabine is either deaminated by deoxycytidine deaminase into an inactive metabolite, 2′,2′ difluoro deoxy uridine (dFdU), or phosphorylated by deoxycytidine kinase into its active form, dFdC monophosphate (dFdCMP). This metabolite is subsequently converted into diphosphate and triphosphate derivatives, which incorporate into DNA and cause chain termination. Although gemcitabine shares structural and mechanistic similarities with cytarabine (Ara-C), it demonstrates a broader spectrum of antitumor activity (20).

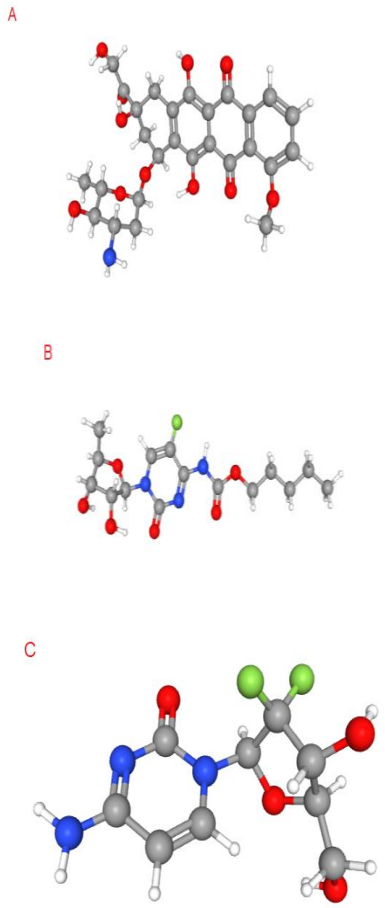

Figure 2. Three-dimensional structures of the drugs Epirubicin, Xeloda, and Gemcitabine. a: Epirubicin b: Xeloda c: Gemcitabine.

Natural Products in Cancer Therapy

Currently, about 75% of clinically used anticancer drugs are derived from natural sources such as plants, animals, and microorganisms (21). Since ancient times, plants have been widely used in traditional medicine for wound healing, disease treatment, and general health maintenance (22).

Formononetin is an O-methylated isoflavone with a molecular weight of 268.268 g/mol (Figure 3.A). It is commonly found in legumes, various clover species particularly red clover (Trifolium pratense L.) and in the traditional Chinese medicinal plant Astragalus membranaceus (Fisch) (23). In cancer cells, including breast cancer, formononetin regulates the balance between pro-apoptotic and anti-apoptotic proteins of the Bcl-2 family. Studies demonstrate that formononetin induces dose-dependent alterations in the

Bax/Bcl-2 protein ratio, promoting apoptosis in tumor cells (24).

Curcumin, a polyphenol extracted from Curcuma species (Figure 3.B), is well known for its broad anticancer activity and its ability to interfere with several hallmarks of tumor progression (25). One key mechanism involves inhibition of the transcription factor NF-κB, which controls processes such as cell proliferation, angiogenesis, metastasis, apoptosis, and resistance to therapy (26). Curcumin suppresses NF-κB activation through inhibition of IκB kinase signaling.

Additionally, curcumin downregulates Cyclin D1, an oncogenic driver of cell cycle progression and proliferation that is often overexpressed in cancers (27,28). Both in vitro and in vivo studies have shown that curcumin enhances TP53 expression and promotes apoptosis (29). It also prevents phosphorylation of retinoblastoma protein (RB), a key tumor suppressor regulating the cell cycle (30). Moreover, curcumin inhibits signaling mediated by epidermal growth factor (EGF) and its receptor EGFR, both of which are frequently overexpressed in breast tumors and contribute to cancer progression (31,32).

Molecular Docking

Molecular docking has become an important tool in modern in-silico drug development. This method predicts how a small molecule interacts with a protein at the atomic level (33). By doing so, it helps researchers analyze how compounds, including nutrients or drugs, bind to target proteins and provides insight into the biochemical processes involved (34). Docking is a structure-based approach, which means it requires a high-resolution three-dimensional structure of the target protein. Such structures are usually obtained through X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, or cryo-electron microscopy (35-37).

In this study, we aim, for the first time, to use molecular docking to compare the binding affinities of commonly used breast cancer drugs, including Epirubicin, Xeloda and Gemcitabine, alongside two plant-derived compounds, formononetin and curcumin, to two key proteins, DLAT and ATOX1. This innovative approach allows for the identification of potential interactions between conventional drugs and plant compounds with critical molecular targets in breast cancer, providing new insights for the development of combination therapies.

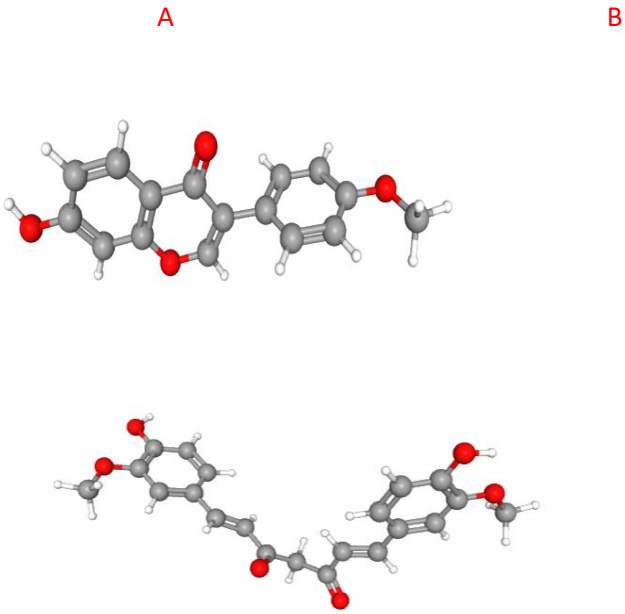

Figure 3. Three-dimensional structures of formononetin and curcumin. a: Formononetin. b: Curcumin.

Material and methods

To perform the docking process, we need the three-dimensional structure of proteins, drugs, and plant compounds. First, we entered the PDB site at rcsb.org and searched for the name of the Atox1 protein in the search section and saved the desired protein in the PDB format. We did the same for the DLAT protein. Then, with the help of the pubchem site at pubchem.ncbi.nlm.nih.gov, we saved the three-dimensional structure of the drugs Epirubicin, Xeloda, and Gemcitabine and the plant compounds Formononetin and Curcumin in the SDF format. We downloaded the protein structures from the PDB database and used Chimera software version 1.8 to remove impurities and prepare the proteins for docking. Since the DLAT protein consists of three chains, we selected the A chain, which was larger, and deleted the

other chains using the software's select option, selecting chain, then the action option, selecting the atom binding option, and the delete option. The Atox1 protein also consists of two chains, which we selected chain B and deleted chain A. To make modifications, including adding hydrogen ions and charge flow, removing excess molecules and water from the selected chain, we used the Tools option, then selected the Surface Binding Analysis option, then the Dock Prep option, and checked all the desired changes from the opened menu. After confirming the protein structure, it is ready for docking (Figure 3). Then we saved the proteins in PDB format, and in the next step, we loaded the proteins individually into the Pyrx software version 0.8 and defined the protein as a macromolecule in the software. For this, we used the File option, then the Load Molecule option. When the name and shape of the protein appeared in the software, we clicked on the protein name and selected Autodock Make Macromolecule, and then loaded the desired drugs and herbal compounds one by one in the SDF format into the Pyrx software as ligands. For this, we used the File option and then the Import option. In order to apply the changes made to the protein to the drug, we clicked on the molecular formula of the drug or herbal compound that we entered into the software and selected the convert to PDBQT option. After minimizing, we docked the protein and drug and set the desired coordinates for the drug-protein binding so that the center of the search grid for the DLAT protein was defined at X: 59.5815, Y: 28.7394, Z: 65.9339 and for the ATOX1 protein at X: 33.8357, Y: 25.5754, Z: 39.585, and the Forward option was executed. After a few minutes, the software provided a table of binding affinity values. The interpretation of the results was as follows: the more negative the binding affinity value (for example, less than -5), the stronger the binding between the drug and the protein. No organic ligands or crystallized metal ligands suitable for rebinding validation are available for ATOX1 (PDB ID: 1FE0) and DLAT (PDB ID: 3B8K). Therefore, classical RMSD rebinding could not be performed. The docking protocol for the test compounds was set up using AutoDock Vina implemented in PyRx with the following standard settings: The binding affinity was calculated using the AutoDock Vina scoring function, which estimates the binding free energy (kcal/mol) based on the experimental interaction terms. All ligands were protonated assuming physiological pH (7.4) during PDBQT transformation using Open Babel implemented in PyRx, and the exhaustiveness parameter was set to 8 to ensure adequate structure sampling.

Protein-ligand interaction analysis was performed only for the DLAT protein and the three ligands that showed the highest binding affinity. This was done with Chimera software version 1.8. Hydrogen bonds were identified using the FindHBond option and proximity-based contacts were identified using the Contacts option with a distance limit of 4.0 Å. Interaction analysis was restricted to selected protein-ligand complexes to allow for a focused interpretation of the most stable binding states.

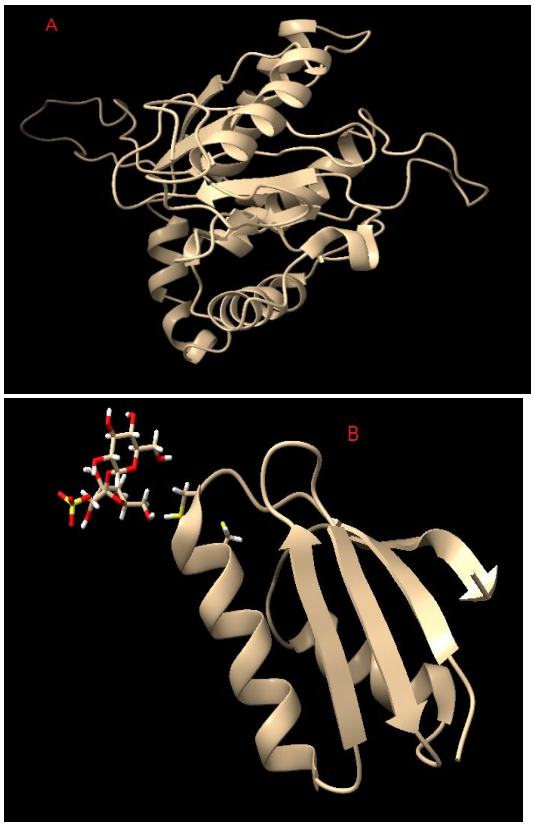

Figure 4. Proteins DLAT and Atox1 after modifications in Chimera software. a. Chain A of the DLAT protein. b. Chain B of the Atox1 protein.

Results

The results of docking between the protein ATOX1 and the drug Xeloda are presented in Table 1. Similarly, the

results of docking between the protein DLAT and the drug Xeloda are presented in Table 2.

Table 1. Results of molecular docking between ATOX1 protein and the drug xeloda.

Mode	RMSD Upper (ATOX1)	RMSD Lower (ATOX1)	Affinity (ATOX1)
0	0.000	0.000	-5.1
1	9.225	7.970	-5.0
2	10.022	8.181	-4.9
3	10.495	3.837	-4.9
4	9.928	8.216	-4.9
5	8.775	3.332	-4.7
6	8.771	5.313	-4.6
7	20.698	18.336	-4.6
8	9.138	2.439	-4.6

Table 2. Results of molecular docking between DLAT protein and the drug xeloda.

Mode	RMSD Upper (DLAT)	RMSD Lower (DLAT)	Affinity (DLAT)
0	0.000	0.000	-7.5
1	2.314	1.517	-6.6
2	22.744	21.020	-6.5
3	2.004	1.426	-6.5
4	2.701	1.660	-6.4
5	20.706	19.497	-6.1
6	21.826	20.831	-6.0
7	23.079	21.243	-6.0
8	2.828	1.644	-6.0

The results of the docking between the protein ATOX1 and the drug Gemcitabine, are presented in Table 3. Similarly, the results of the docking between the protein DLAT and the drug Gemcitabine, are presented in Table 4.

Table 3. Results of molecular docking between ATOX1 protein and the drug gemcitabin.

Mode	RMSD Upper (ATOX1)	RMSD Lower (ATOX1)	Affinity (ATOX1)
0	0.000	0.000	-5.4
1	4.557	2.911	-5.0
2	4.346	2.394	-4.9
3	4.909	3.100	-4.9
4	1.770	1.593	-4.6
5	12.535	11.476	-4.4
6	3.382	2.094	-4.3
7	14.778	13.508	-4.2
8	13.357	11.841	-4.1

Table 4. Results of molecular docking between DLAT protein and the drug gemcitabin.

Mode	RMSD Upper (DLAT)	RMSD Lower (DLAT)	Affinity (DLAT)
0	0.000	0.000	-6.5
1	7.023	4.484	-6.3
2	5.501	3.684	-6.2
3	6.280	4.225	-5.9
4	5.262	2.984	-5.8
5	21.518	20.352	-5.8
6	21.356	20.089	-5.7
7	21.436	20.285	-5.6
8	20.689	19.633	-5.3

The results of the docking analysis between the DLAT protein and the drug Epirubicin, are presented in Table 5. Similarly, the results of the docking analysis between the ATOX1 protein and the drug Epirubicin, are presented in Table 6.

The results of docking between the ATOX1 protein and formononetin, are presented in Table 7. Similarly, the results of docking between the DLAT protein and formononetin, are presented in Table 8.

Table 5. Results of molecular docking between DLAT protein and the drug epirubicin.

Mode	RMSD Upper (DLAT)	RMSD Lower (DLAT)	Affinity (DLAT)
0	0.000	0.000	-5.8
1	5.599	2.871	-5.5
2	14.370	10.963	-5.3
3	5.193	2.509	-4.7
4	6.813	2.543	-4.6
5	17.697	13.946	-4.6
6	13.138	9.389	-4.5
7	16.923	13.055	-4.3
8	16.092	12.095	-4.1

Table 6. results of molecular docking between ATOX1 protein and the drug epirubicin.

Mode	RMSD Upper (ATOX1)	RMSD Lower (ATOX1)	Affinity (ATOX1)
0	0.000	0.000	-5.6
1	12.553	7.619	-5.5
2	11.019	7.183	-5.4
3	6.102	2.689	-5.4
4	12.283	7.657	-5.3
5	7.476	4.337	-5.3
6	11.138	8.349	-5.3
7	5.127	2.371	-5.2
8	5.802	3.213	-5.1

Table 7. Results of molecular docking between ATOX1 protein and the formononetin.

Mode	RMSD Upper (ATOX1)	RMSD Lower (ATOX1)	Affinity (ATOX1)
0	0.000	0.000	-5.4
1	1.886	0.751	-5.1
2	10.795	8.228	-5.0
3	10.62	9.054	-4.9
4	10.746	8.58	-4.9
5	2.243	2.096	-4.9
6	12.325	9.354	-4.7
7	12.734	9.801	-4.6
8	14.531	13.34	-4.6

Table 8. Results of molecular docking between DLAT protein and the formononetin.

Mode	RMSD Upper (DLAT)	RMSD Lower (DLAT)	Affinity (DLAT)
0	0.000	0.000	-8.3
1	2.328	1.376	-8.3
2	3.664	2.499	-7.5
3	22.949	20.777	-6.2
4	23.25	21.161	-6.2
5	19.917	18.061	-6.0
6	23.187	20.948	-6.0
7	22.114	20.95	-5.7
8	18.633	16.007	-5.6

The results of the docking analysis of the ATOX1 protein with curcumin, are presented in Table 9. Similarly, the results of the docking analysis of the DLAT protein with curcumin, are presented in Table 10.

Table 9. Results of molecular docking between ATOX1 protein and the curcumin.

Mode	RMSD Upper (ATOX1)	RMSD Lower (ATOX1)	Affinity (ATOX1)
0	0.000	0.000	-5.8
1	11.602	7.607	-5.8
2	11.701	7.624	-5.8
3	12.949	9.714	-5.7
4	8.481	3.969	-5.6
5	12.788	8.622	-5.5
6	9.699	2.98	-5.4
7	10.756	6.818	-5.4
8	10.411	6.834	-5.4

Table 10. results of molecular docking between DLAT protein and the curcumin.

Mode	RMSD Upper (DLAT)	RMSD Lower (DLAT)	Affinity (DLAT)
0	0.000	0.000	-8.2
1	7.74	0.595	-8.1
2	22.028	20.25	-6.8
3	22.669	20.116	-6.7
4	22.017	20.241	-6.7
5	22.208	20.36	-6.6
6	7.523	2.398	-6.6
7	22.002	20.042	-6.6
8	23.197	20.967	-6.2

Residue-level contact analysis between the DLAT protein and formononetin, curcumin, and Xeloda revealed that all three ligands interacted with DLAT primarily through hydrophobic and van der Waals interactions, and no conventional hydrogen bonds were identified in any of the complexes (Table 11). Curcumin exhibited the most compact binding mode, forming frequent short-range contacts with ALA408, ALA499, and ILE493, indicating tight packing within a hydrophobic compartment of DLAT (Figure 5.B). In contrast, Xeloda formed a more extensive network of contacts involving both hydrophobic and polar residues such as CYS402, THR476, and ARG535, indicating a less compact but still stable binding pattern (Figure 5.A). Formononetin interacted primarily with aromatic residues including PHE155 and TRP223, consistent with π-π stacking-based stabilization rather than insertion into the deep cavity (Figure 5.C).

Table 11. Residue-level contact analysis of DLAT with curcumin, Xeloda, and formononetin.

Ligand	Binding Affinity	Key DLAT residues involved	Dominant interaction types	Distance range (Å)	Binding interpretation
Curcumin	-8.2	ALA408, ALA499, ILE493, THR476, CYS402, ASP533, PHE477	Hydrophobic and van der Waals contacts (no hydrogen bonds detected)	1.50–3.77	Compact accommodation of curcumin within a predominantly hydrophobic DLAT pocket
Xeloda (Capecitabine)	-7.5	CYS402, ALA408, ALA499, THR476, ASP533, PHE477, ARG535, ILE493	Mixed hydrophobic and polar contacts (no hydrogen bonds detected)	1.47–3.58	Stable but more distributed interactions compared to curcumin
Formononetin	-8.3	PHE155, CYS80, GLU85, THR154, LEU208, TRP223, VAL83, ALA86	Hydrophobic and aromatic (π–π) contacts (no hydrogen bonds detected)	1.78–3.77	Aromatic anchoring through phenylalanine and tryptophan residues

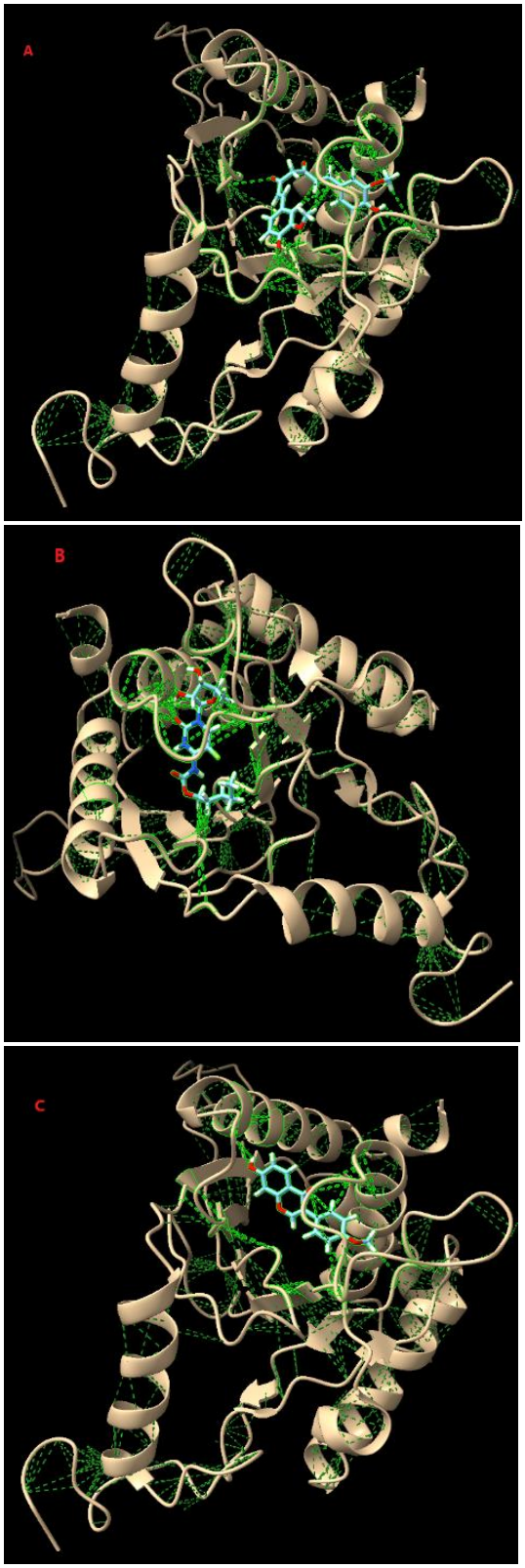

Figure 5. 3D representation of the binding of A: Curcumin, B: Xeluda, and C: Formononetin within DLAT. DLAT is shown as a cartoon, while the ligands are shown as sticks. Proximity-based contacts are highlighted with green dashed lines. No classical hydrogen bonds were identified in any of the complexes.

Discussion

In this study, the interactions of the anticancer drugs epirubicin, Xeloda, and gemcitabine, as well as the natural compounds curcumin and formononetin, with the human proteins DLAT and ATOX1 were investigated using molecular docking. According to the results obtained from the PyRx software, more negative binding values indicate stronger interactions between the compound and the protein. The findings showed that all three studied drugs were able to bind to both proteins, but their binding intensity was higher with DLAT. Among the drugs, Xeloda, with a binding affinity of -7.5 kcal/mol, showed the strongest effect and binding strength on DLAT, indicating the high potential of this drug to target DLAT in the treatment of breast cancer. This result emphasizes the importance of DLAT as a therapeutic target in breast cancer. The natural compounds formononetin and curcumin also showed strong binding to both proteins, with stronger interactions with DLAT (formononetin: -8.2 kcal/mol, curcumin: -8.3 kcal/mol), surpassing the tested drugs. The binding of formononetin to DLAT appears to be stabilized primarily by aromatic and hydrophobic interactions with residues including PHE155 and TRP223, suggesting that π–π stacking rather than hydrogen bonding plays a dominant role in complex formation. These results suggest that these natural compounds can exert significant inhibitory effects on DLAT and may provide a basis for future experimental studys. According to the research of Xiao Qin et al., curcumin bound to ATOX1 (score = -6.1 kcal/mol) and reduced the levels of ATOX1, ATP7A, and COX17 proteins in NSCLC cells (38). These results are consistent with our data (score = -5.8 kcal/mol), the binding of the studied drugs and compounds to ATOX1 was acceptable, although less than DLAT, indicating that DLAT is the main and more effective target of these compounds. Formononetin, xeloda, epirubicin and gemcitabine have not been directly investigated and docked with ATOX1 or DLAT in published articles so far, our analyses can be the basis for future studies. Comparison with previous studies suggests that curcumin and formononetin exert anticancer effects by inhibiting key proteins such as DLAT, which is consistent with our data. These findings support the notion that natural compounds may enhance therapeutic efficacy when combined with

chemotherapeutic drugs. Overall, the results of this study suggest that DLAT could be an important molecular target for breast cancer treatment and that natural compounds have stronger inhibitory potential. These findings may provide a basis for further experimental studies and the design of combination therapies.

Conclusion

The results of this study indicate that plant-derived compounds have significant potential as anticancer agents and can be used in combination with chemical drugs to improve therapeutic efficacy. Molecular docking software assigns a score to each ligand-protein binding state, which indicates the strength and stability of the interaction. This information can facilitate the design of targeted drugs and the selection of more effective treatments.

The use of molecular docking in the design of new drugs is very valuable, as it can reduce the time and resources required for drug development and testing. This technique also allows pharmaceutical companies to bioinformatically evaluate drug-receptor interactions before production and clinical testing, and to benefit from active plant compounds in the development and design of new drugs.

In addition, molecular docking also allows the evaluation of the potential of existing drugs that are not currently used for cancer; a topic that has attracted the attention of researchers in recent years. However, docking results need to be confirmed by laboratory experiments, and the simultaneous use of natural compounds with breast cancer chemotherapy drugs requires further evaluation and investigation.

Author contribution

Conceptualization: **FA**. Data curation: **FF**. Formal analysis: **FA**, **FF**. Methodology: **FA**, **FF**. Software: **FA**, **FF**. Supervision: **FA**. Validation: **FF**. Visualization: **FA**, **FF**. Writing original draft: **FF**. Writing review & editing: **FA**, **FF.**

Conflicts of interest

There are no conflicts of interest.

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