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An in-silico approach to target Breast cancer with novel phytochemicals

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Abstract

Aging leads to the manifestation of various diseases. Cancer being one of the major illness that is associated with the age. Breast cancer is still a relatively global health concern as the leading cancer cause of death among women. The current study also utilizes an in-silico approach to discover and characterize new phytochemicals as potential therapeutic agents to breast cancer. Here, we explore the molecular basis driving the makeup of breast cancer, with an emphasis on the PI3K/AKT/mTOR pathway, a well-known signaling cascade implicated in cell growth and therapy response. Two candidates (Thalidasine (-8.80 Kcal/mol) and 7-Epiclusianone (-8.62 Kcal/mol)) were found to be promising leads displaying high binding affinity and therapeutic potential through screening a diverse library of phytochemicals against key breast cancer target proteins. These phytochemicals exhibit diverse antineoplastic effects as cell proliferation, angiogenesis, and metastasis inhibitors, and/or as inducers of apoptosis. Besides, their ability to specifically target breast cancer stem cells and to increase sensitivity to traditional therapies make them an appealing option for breast cancer adjuvant therapy. Taken together our data suggest that these phytochemicals should be further studied alone and in novel nano formulations that enhance bioavailability and reduce toxicity. This study adds to the evidence for the potential role of natural products in breast cancer and the role of lifestyle in cancer prevention.

Keywords: Phytochemicals; Molecular Docking; Breast Cancer; *In-Silico*

1. Introduction

Aging is a complex irreversible change leading to decreased muscle health and increase in the manifestation of diseases. Various diseases such as Cancer, Parkinson, Alzheimer and infectious disease increase with increase in age. Aging and Cancer is a prominent global health concern, accounting for approximately one in six deaths worldwide (Bray et al., 2018). It is often divided into benign and malignant tumours and shows up as unchecked cell proliferation. Benign tumours develop slowly and have distinct borders, whereas malignant tumours proliferate quickly and have erratic borders (American Cancer Society, 2020). Breast and cervical cancers account for a substantial portion of women's death rates, with over fifty forms discovered (Ferlay et al., 2020). The most prevalent cancer in women between the ages of 45 and 65 is breast cancer, which frequently starts as ductal carcinoma in situ (DCIS) and can spread to other areas if the basement membrane is compromised (Haffty et al., 2020). Hereditary breast cancer risk is significantly influenced by genetic predispositions, specifically mutations in BRCA1 and BRCA2. Genetic predispositions are a major factor in the risk of hereditary breast cancer, especially mutations in BRCA1 and BRCA2 (King et al., 2003). The growth and survival of cancer cells depend on important signalling pathways, most notably the PI3K/AKT/mTOR pathway, and changes to this route frequently result in treatment resistance (Mora et al., 2014). New targeted treatments, such as HDAC and PI3K inhibitors, have the potential to reduce toxicity while increasing therapeutic effectiveness (Zhao et al., 2020; Nishijima et al., 2019). Significant progress has been made in understanding the molecular causes of breast cancer, which now includes the function that oestrogen and its receptor play in stimulating

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tumour growth. Estrogen's pro-growth actions account for over 70% of cases of breast cancer, with different subtypes distinguished by the presence or lack of HER2 receptors, progesterone, and oestrogen (Yip et al., 2021).

Since medicines can be customised to target particular hormonal pathways, this receptor-driven classification has significant medical implications. Treatment strategies have been transformed by the interaction between tumour suppressor genes and oncogenes, which allows for targeted medicines that interfere with particular cellular pathways. Furthermore, preclinical research has shown that PI3K inhibitors and conventional chemotherapeutics such anthracyclines work in concert (Tiwari et al., 2022). Research into drug delivery systems mediated by nanoparticles that can improve treatment outcomes while reducing side effects is being driven by issues like cardiotoxicity, which are still major problems (Zhao et al., 2018).

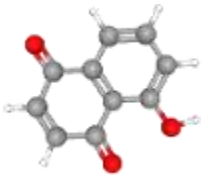
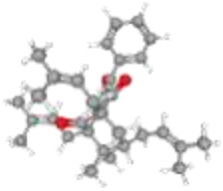
Further highlighting the necessity of comprehensive therapeutic approaches in the fight against this complex disease, research into phytochemicals and traditional remedies like Juglone offers encouraging prospects for novel cancer treatments (Ortega et al., 2020; Hanemann et al., 2020). According to Haanen and Robert (2018), patients with advanced breast cancer now have new hope thanks to recent developments in immunotherapy, especially the use of checkpoint inhibitors, which have broadened the therapeutic options. Developing successful treatment techniques requires an understanding of the tumour microenvironment, which includes fibroblasts, immune cells, and extracellular matrix components (Rosenfeld et al., 2021).

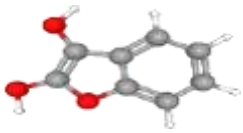
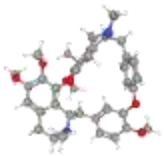
The need for personalised medicine techniques to maximise therapeutic success is highlighted by ongoing research into the genetic variability of tumours (Hyman et al., 2017). Cancer care is beginning to change as a result of the incorporation of artificial intelligence and machine learning into patient data analysis, which makes it possible to create more customised and accurate treatment regimens (Esteva et al., 2019). A multifaceted approach that combines cutting-edge research, novel medicines, and patient-centred care will be essential in tackling this major global health issue as we continue to investigate the complexity of cancer biology. Our analysis will be centred on the potential of particular phytochemicals as therapeutic targets for breast cancer.

2. Material and Methods

Six ligands were chosen for docking, and the structure of the ligands was downloaded from PubChem in 3d conformer format, which will be in an SDF file, in which four of them were phytochemicals Anacardic acid, Dihydroxy benzofuran, 7-epi clusianone, Thalidasine, and one of the ligands was Epirubicin and the last one Juglone. These SDF file ligand structures must be converted into PDB files for docking using the Open Bebel tool. Phytochemicals were chosen for docking to determine the binding energies of the phytochemicals with cancer-inhibiting proteins to determine whether these phytochemicals can be used for the treatment of breast cancer as drugs and their efficacy in cancer cell therapy with less toxicity. Epirubicin is a drug used for breast cancer and is proven to be even more effective when used with the dual inhibitor PI3Ks/HDAC, which is used as a ligand to check its efficacy on the selected proteins of cancer.

Table 1 Ligands used and their structure

Ligands	**Structures**
Juglone	
7-Epiclusianone	

Anacardic acid	
Dihydroxybenzofuran	
Thalidasine	

2.1. Receptor

The 3D PDB file structure of cancer protein HER2+ using PDB ID 4HRL, BCL2 using PDB ID 5UUK, and c-Myc using PDB ID 2okv were downloaded from RCBS (http://www. rcsb. org). The Swiss PDB viewer protocol was optimized for energy minimization by dividing the dedicated ligands (Tripathi and Imran 2020). These proteins have also been associated with cancer cells. The protein selected for docking is a crucial target for research aimed at developing drugs to combat breast cancer. The selected ligands were docked with these proteins to assess their compatibility and gain insights into the interaction of small molecules within the binding site of the target proteins, as well as to comprehend fundamental biochemical processes.

3. Results and Discussion

Gene expression analysis plays a pivotal role in breast cancer research, particularly in the context of drug discovery. Overexpression of specific genes has been implicated in the pathogenesis of breast cancer, with critical roles in cell growth, proliferation, metabolism, and survival. Notably, proteins such as BCL2, HER2, and c-Myc have emerged as key players in the development and progression of this malignancy. The BCL2 gene encodes an important inhibitor of programmed cell death (apoptosis). Its enhanced expression has been linked to increased proliferation of breast cancer cells and has been identified as a potential biomarker for predicting lymph node metastasis in cancer patients (Raha et al., 2022). Conversely, downregulation of BCL2 may facilitate apoptosis and contribute to tumor suppression (Merino et al., 2020). Phytochemicals like Juglone are bonded together to see their binding energy at active sites of the proteins which gives the idea of the efficacy of the complex formed. lowest binding energy of the complex shows a better result and shows the toxicity level of the complex which gives clarity for the drug discovery has been shown to Table no 2.

HER2 (human epidermal growth factor receptor 2) is another crucial protein that, when overexpressed, leads to aggressive tumor growth and increased metastatic potential. HER2-positive breast cancer cells exhibit significantly elevated levels of HER2 receptors, which enhance the tumor's growth capabilities (N & N, 2014). The overexpression of c-Myc, a master regulator of growth and metabolism, triggers numerous cellular changes, including increased proliferation and transcriptional activity, ultimately disrupting normal cell division processes (Takahashi et al., 2023).Phytochemicals, bioactive compounds derived from plants, have garnered attention for their potential anti-cancer properties. Juglone, a natural naphthoquinone found in walnut tree components, has demonstrated efficacy in inducing apoptosis, inhibiting angiogenesis, and preventing cancer cell migration (Wang et al., 2023). Similarly, anacardic acid (AA), derived from Anacardiaceous plants. Inhibit cell growth and promote apoptosis in triple-negative breast cancer (TNBC) cell lines, specifically MDA-MB-231 (Kumar et al., 2022).Benzofuran derivatives are currently under investigation for their diverse pharmacological effects, including anti-tumor activity. Recent studies have synthesized 3-acyl-5-hydroxybenzofuran derivatives, revealing varying antiproliferative effects against human breast cancer cell lines, such as MCF-7 (Smith et al., 2023). Furthermore, 7-Epi Clusianone has shown significant promise in inhibiting cell proliferation and migration across luminal A and claudin-low breast cancer cell lines, demonstrating its potential as a therapeutic agent (Johnson et al., 2024). In our experiments, we analyzed the binding energies of selected ligands, including Thalidasine and 7-Epiclusianone, with the cancer-associated proteins BCL2, HER2, and c-Myc. Notably, Thalidasine and 7-Epiclusianone exhibited the lowest binding energies among the ligands tested, suggesting a

stronger affinity for these critical proteins. This low binding energy correlates with enhanced efficacy and provides valuable insights into the potential toxicity levels of the drug candidates, guiding future drug development efforts.

Table 2 The unique identifier for the protein structure in the Protein Data Bank (5UUK) Binding Energy, favourable interaction, H-bond with *In-silico* 3-D interaction with Juglone, Anacardic acid, Thalidasine , 7-Epiclusianone and Dihydroxybenzofuran

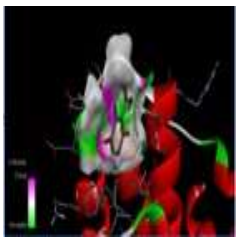
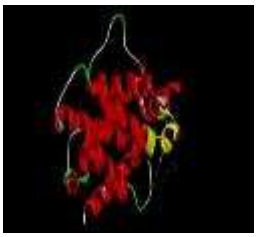
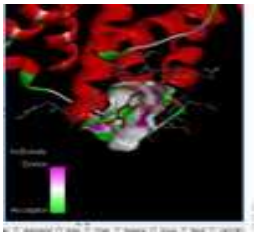
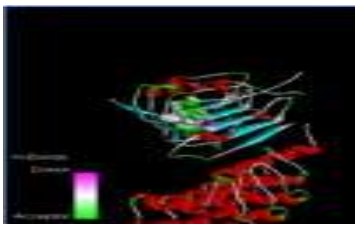
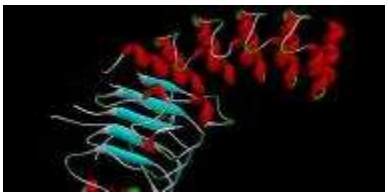
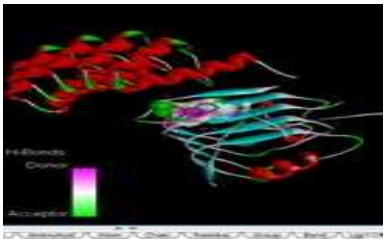
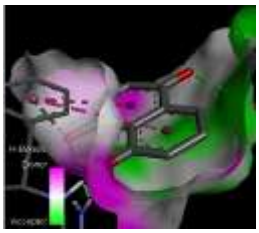
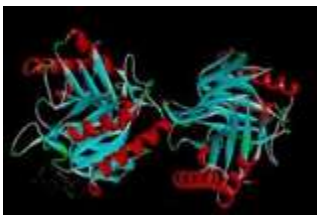
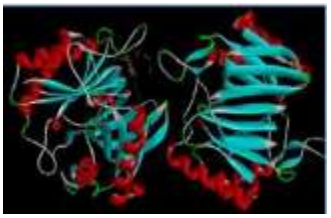
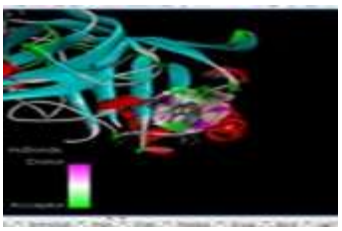
PDB ID	LIGANDS	IMAGE	HYDROGEN BONDS	ACTIVE SITE
5uuk	Juglone		2	GLN A:107
	Anacardic acid		0	0
	Thalidasine		0	0
	7-Epiclusianone		0	0
	Dihydroxybenzofuran		3	GLN A:107

Table 3 The unique identifier for the protein structure in the Protein Data Bank (4HRL) Binding Energy, favourable interaction, H-bond with *In-silico* 3-D interaction with Juglone, Anacardic acid, Thalidasine , 7-Epiclusianone, Dihydroxybenzofuran and Epirubicin.

PDB ID	LIGANDS	IMAGE	HYDROGEN BONDS	ACTIVESITE
4HRL	Juglone		3	LEU C:123
	Anacardic acid		0	0
	Thalidasine		0	0
	7-Epiclusianone		0	0
	Dihydroxybenzofuran		0	LEU C:85 ASP C:88
	Epirubicin		0	0

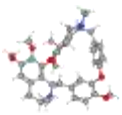
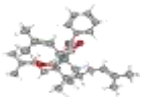
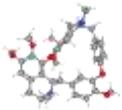
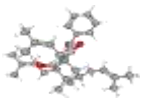
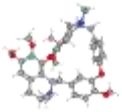
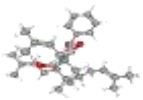
There are various gene of breast cancer present; ATM, BARD1, BRIP1, CASP8, CTLA4, CYP19A1, FGFR2, H19, LSP1, MAP3K1, MRE11A, NBN, RAD51, and TERT etc. Genes selected for the experiment are c-Myc, Bcl2, and Her2+.Overexpression of genes are responsible for causing cancer, these genes are docked with the phytochemicals which are taken by the ADMET study. Phytochemicals which are taken are Juglone, Dihydroxybenzofuran, Thalidasine, Anacardic acid and 7-Epiclusianone and Epirubicin as a control.

Table 4 The unique identifier for the protein structure in the Protein Data Bank (2OKV) Binding Energy, favourable interaction, H-bond with *In-silico* 3-D interaction with Juglone, Anacardic acid, Thalidasine , 7-Epiclusianone, Dihydroxybenzofuran and Epirubicin.

PDB ID	LIGANDS	IMAGE	HYDROGEN BONDS	ACTIVE SITE
2OKV	Juglone		0	ALA
				B:129
				GLN
				B:79
				PHE B
				:80
	Anacardic Acid		0	0
	Thalidasine		0	0
	7-Epiclusianone		0	0
	Dihydroxy		2	LEU
	Benzofuran			D:31
				ILE
				D:33
	Epirubicin		0	0

Phytochemicals taken showed good binding energy and hydrogen bonds. Epirubicin is a well formulated drug for breast cancer is taken as control to compare the efficacy of the phytochemicals with it. The selected genes are identified using bibliographic study. Further on screening for various phytochemicals these five phytochemicals are selected for the docking with the genes. Owing to the binding affinity of score of 6 phytochemicals the top selected 2 phytochemicals with each receptor are chosen has been shown to table 5

Table 5 The unique identifier for the protein structure in the PDB ID (5UUK, 4HRL and 2OKV) Binding Energy, favourable interaction on number of runs with *In-silico* 3-D interaction with Juglone, Anacardic acid, Thalidasine , 7-Epiclusianone, Dihydroxybenzofuran and Epirubicin.

SR.NO.	PDBID	LIGAND	STRUCTURE	RUN	BINDING ENERGY
1.	5UUK	Thalidasine		3	-7.78
		7-Epiclusianone		9	-7.61
2.	4HRL	Thalidasine		2	-8.80
		7-Epiclusianone		3	-8.61
3.	2OKV	Thalidasine		5	-9.51
		7-Epiclusianone		7	-8.61

4. Conclusion

Our screening of various phytochemicals against key breast cancer target proteins has identified two highly promising therapeutic candidates i.e Thalidasine (-8.80 Kcal/mol) and 7-Epiclusianone (-8.62 Kcal/mol). We believe these findings can contribute significantly to traditional medicine approaches and assist in discovering viable leads for optimizing breast cancer treatments. Previous studies have shown that these phytochemicals can effectively suppress gene overexpression linked to breast cancer. This research aims to identify suitable and potent bioactive compounds through a comprehensive bioinformatics approach, ultimately leading to effective breast cancer therapies. The phytochemicals assessed exhibit anti-cancer properties through multiple mechanisms, including the inhibition of cell proliferation, angiogenesis, migration, invasion, and metastasis, along with the induction of cell cycle arrest and apoptosis via modulation of various genes and signaling pathways. Moreover, they have the potential to target breast cancer stem cells, combat drug resistance, and enhance sensitivity to radiation. There is growing interest in natural remedies that may mitigate the side effects associated with conventional cancer treatments. Many phytochemicals are abundant in common foods such as vegetables, grains, and fruits, and their regular consumption could be an effective strategy for breast cancer prevention. It is estimated that lifestyle changes could reduce cancer incidence by up to two-thirds, highlighting the importance of public awareness regarding the benefits of phytochemical consumption. While our understanding of multistage carcinogenesis has improved, the mechanisms by which many phytochemicals exert their anticancer effects remain inadequately understood.

Some phytochemicals are found in limited quantities in wild plants, complicating their collection for therapeutic use. In such cases, elicitation strategies and plant tissue culture techniques offer valuable insights for enhancing the accumulation of cancer-related compounds and conserving the respective plant species. Additionally, an in silico

approach plays a crucial role in identifying and optimizing phytochemicals with anticancer properties, enabling researchers to predict their interactions with biological targets and assess their efficacy before moving to experimental stages. Such formulations may facilitate sustained release of the compounds, effectively addressing issues related to drug resistance and reducing the adverse effects commonly associated with traditional chemotherapeutics. This integrated approach not only optimizes the therapeutic efficacy of phytochemicals but also paves the way for innovative treatments in breast cancer care.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author's contribution

- **Deepika Dwivedi, DD**- Data curation, methodology and software supervision, writing original draft.
- **Ishani Krishnawat, IK-** Data curation and methodology.
- **Meenakshi Sharma, MS**- Conceptualisation, supervision, validation, finalizing, reviewing and editing of the final manuscript.

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