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INTEGRATING BIOINFORMATICS TOOLS; MOLECULAR SCREENING FOR DRUG DISCOVERY AGAINST ESTROGEN POSITIVE BREAST CANCER

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Abstract
Breast cancer is the most devastating malignancy affecting women globally. Although effective treatment options are available, many of these drugs can cause severe side effects and may be ineffective in some patients, leading to limited therapeutic outcomes. This study aims to identify potent bioactive compounds through extensive screening of various phytochemicals using bioinformatics techniques to develop a promising therapeutic strategy for estrogen positive breast cancer. The study utilized breast cancer-related estrogen protein and analyzed Nigella sativa phytochemicals with established anticancer properties based on their pathophysiological relevance, pharmacokinetics, and drug-like characteristics using the SwissAdme web server. After conducting molecular docking with p, the top six ligands for protein were identified. The selected compounds were further evaluated for bioavailability and toxicity using SwissAdme and ADMETSAR to ensure their potential as viable therapeutic candidates. The initial screening of 60 phytochemicals identified 9 compounds that complied with the Lipinski rule. Based on their binding affinity scores, the top six molecules were selected, leading to a shortlist of 6 potential drug candidates. These compounds Thymoquinone, Gallic acid, Apigenin, Luteolin, Patuletin and Myricetin were found to be safe and non-toxic. The findings contribute to advancing traditional medicine-based therapies and identifying potential candidates for lead optimization in breast cancer treatment. These results can be validated through molecular dynamics simulations and experimental studies using animal models, paving the way for the development of targeted breast cancer therapies.

Introduction
Cancer is a devastating disease marked by the unregulated proliferation of aberrant cells, which have the capacity to invade and metastasize to distant tissues and organs[1]. Breast cancer is the most prevalent form of metastatic cancer in women and ranks among the primary contributors to illness

and death on a global scale[2] Breast cancer accounts for 31% of all malignancies in women, while lung cancer, colorectal cancer, and breast cancer together comprise 52% of all new diagnoses in female patients[3]. Consequently, despite significant progress in detection and treatment, the mortality rate continues to increase. This challenge arises from the limitations associated with traditional chemotherapy, such as systemic toxicity, low specificity, and high resistance rates, which ultimately restrict clinical responses[4].

Plants have proven to be valuable sources of therapeutic compounds that have significantly contributed to the creation of various modern pharmaceuticals. Their medicinal properties arise from a complex array of chemical constituents, including diverse phytochemicals like terpenoids, alkaloids, flavonoids, phenols, glycosides, and numerous other secondary metabolites[5]. Medicinal plants are rich sources of bioactive compounds that exhibit anti-cancer potential by acting on various biological pathways and mechanisms to combat cancer[6]. Despite their promising potential in cancer treatment, standardization and clinical validation of phytochemicals remain challenging. Addressing these issues requires collaborative research efforts across multiple disciplines to fully harness their therapeutic benefits[7]. Phytochemicals have shown efficacy against anaplastic thyroid, breast, and other cancers by reducing tumor aggressiveness and serving as powerful anti-cancer agents[8].

Nigella sativa, commonly known as black cumin, is a widely utilized medicinal herb that has a long-standing history in traditional medicine. Often referred to as a "miracle herb," N. sativa is reputed for its diverse therapeutic properties, including its potential to combat various diseases, such as cancer. Its anti-cancer effects have been linked to multiple mechanisms, including strong anti-proliferative, pro-apoptotic, antioxidant, anti-mutagenic, and anti-metastatic activities. Additionally, N. sativa demonstrates a capacity to inhibit tumor development and progression by modulating inflammatory responses and enhancing immune function. The herb has shown the ability to increase natural killer (NK) cell activity against cancer cells and regulate key signaling pathways, including p53, and caspases, thereby inhibiting tumorigenesis and supporting anti-cancer defenses[9, 10]

Traditional drug discovery methods are time-intensive, costly, and laborious, often taking up to 12 years and costing 2.7 billion USD. Additionally, current breast cancer treatments may lack sustained effectiveness and pose challenges like side effects and resistance. Computational approaches, however, enable rapid, cost-efficient screening of numerous compounds, providing preliminary data to guide further testing and improving study success rates[11].

Computer-Aided Drug Discovery (CADD) methodologies have been extensively utilized to enhance the efficiency of the drug development process. These techniques streamline drug research by targeting specific systems and objectives, optimizing the overall drug discovery pipeline[12]. Molecular docking is a widely utilized approach in drug discovery to investigate how molecules interact with specific target proteins, aiding in the understanding of their binding behavior and potential efficacy.[13]

Considering the significant roles of key receptors such as ERα (Estrogen Receptor) in the onset and progression of breast cancer, a set of 60 bioactive compounds from black cumin was identified for their potential anticancer properties. The objective of this study is to identify potential bioactive compounds of black cumin by screening through a bioinformatics approach to develop an effective treatment for breast cancer.

Materials and Methods

3.4.1. Protein Identification and Preparation

The three dimensional (3D) structure of Protein was taken from the PDB database Protein Data Bank (https://www.rcsb.org/) PDB ID 3ERT. Protein tertiary structure preparation is a precondition for

computational biology procedures that involve converting macromolecular structures into more amenable docking forms. As a result, the selected protein molecules were first optimized using the protein preparation wizard in discovery studio.

3.4.2. Ligand retrieval and preparation

A library of 60 compounds were selected from the *Nigella sativa* Linn. for in silico screening. The All 2D structures were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). After that, the structures of all phytochemicals were created and prepared for docking using Pyrex. All water and ligand molecules are removed from protein using Discovery Studio.

Drug-Likeness and Toxicity Predictions

The drug ability of compounds were evaluated following Lipinski's or Pfizer rule or [14, 15].

3.4.1 ADMET Properties

The resources that are accessible online are admetSAR and Swiss ADME were employed to assess and investigate the pharmacokinetics—that is, the absorption, distribution, metabolism, excretion, and toxicity of potential medications in the human body [16].

3.4.3. Molecular Docking Analysis

PyRx 0.8 version software was used to perform the docking analysis [17]. Using Pyrex and Discovery studio, receptors and proteins were converted to PDBQT format. Discovery studio was utilized to visualize the interactions between ligand and protein receptor. Docking was controlled by the system command prompt [18].

Results

Drug Screening

Using molecular operating environment (MOE15) [19], 9 compounds were screened from the library of 60 compounds. The best-interacting ligand and receptor were selected based on the S-score and binding interactions shown in Table 1. Each ligand showed varied binding scores and amino acid interactions with Era receptor.

Table 1 Screened compounds from Nigella Sativa compound library

Sr.	Ligand	Pubchem id	Receptor	S-score	Rmsd
1.	Patuletin	5281678	Estrogen	-10.7	2.8
2.	Myricetin	5281672	Estrogen	-10.3	1.4
3.	Rutin	5280805	Estrogen	-9.58	1.9
4.	Apigenin	5280443	Estrogen	-9.2	1.04
5.	Gallic acid	370	Estrogen	-8.8	1.7
6.	Thymoquinone	10281	Estrogen	-8.6	0.47
7.	Luteolin	5280445	Estrogen	-8.6	0.98
8.	Triptolide	107985	Estrogen	-8.6	1.9
9.	stigmasterol	5280794	Estrogen	-8.4	0.93

4.3.2 Drug ability and ADMET profiling

The drug ability of a particular compound can be checked using Lipinski's rule of five by studying their pharmacokinetics (Arif *et al*., 2021). Out of 9 compounds screened as top were analyzed for drug likeness's, and which fulfilled this criterion of being good candidate drugs according to Lipinski's RO5 are shown in table 2, Only six compounds were selected for further docking analysis for being suitable drug candidate on basis of binding energy, RMSD value, pharmacokinetic parameter, and ADMET profiling.

Table 2 Top compounds and their pharmacokinetic parameters

Drug Likeness by SwissAdme (Lipinski Rule of Five)

Sr.	Compound	Molecular Weight g/mol	Nortb	HBD	HBA	Log	A	Violation
1	Thymoquinone	164.20	1	0	2	1.67	47.52	0
2	Gallic acid	170.12	1	4	5	0.50	39.47	0
3	Apigenin	270.24	1	1	6	1.10	88.54	0
4	Luteolin	286.24	1	1	1	2.82	48.01	0
5	Patuletin	332.26	0	0	1	2.71	47.80	0
6	Myricetin	318.23	1	1	1	2.82	48.01	0

Mol. WT; Molecular Weight, **HBD;** Hydrogen Bond Donor, **HBA;** Hydrogen Bond Acceptor, **nortb;** Number of Rotatable Bonds, **LogP;** octanal water partition co-efficient **A;** molar refractivity

Table 3 ADMET profiling of top selected phytochemicals by AdmetSAR

Absorption						
Models	**Thymoquinone**	**Gallic acid**	**Apigenin**	**Luteolin**	**Patuletin**	**Myricetin**
Blood-Brain Barrier	+	-	+	+	+	+
Human Intestinal Absorption	+	+	+	+	+	+
Caco-2 Permeability	+	-	+	+	+	+
P-glycoprotein Substrate	-	-	+	-	-	-
P-glycoprotein Inhibitor	+	-	+	-	-	-
Metabolism						
CYP1A2 inhibition	-	-	-	+	-	+
CYP2C19 inhibition	-	-	-	-	-	-
CYP2C9 inhibition	-	-	-	-	-	-
CYP2C9 substrate	-	-	-	-	-	-
CYP2D6 inhibition	-	-	-	-	-	-
CYP2D6 substrate	-	-	-	+	-	+
CYP3A4 inhibition	-	-	-	-	-	-
CYP3A4 substrate	-	-	+	-	+	-
CYP inhibitory promiscuity	-	-	-	-	-	-
Toxicity						
AMES mutagenesis	-	-	-	-	-	-
Carcinogenicity (binary)	-	-	-	-	-	-

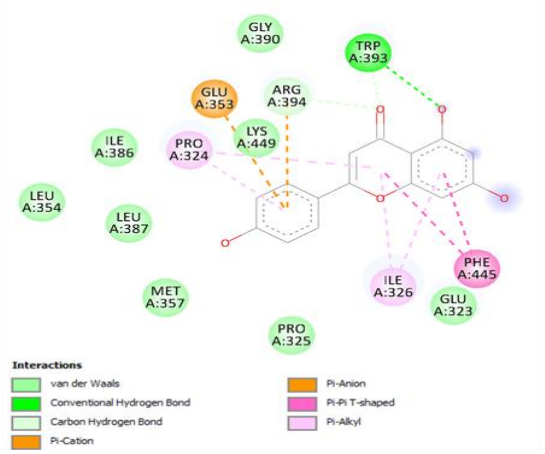
BBB; blood brain barrier, **HIA;** human intestinal absorption, **PGS;** polygalacturonases, **PGI;** phospho-glucose isomerase

4.3.3 Molecular Docking Analysis

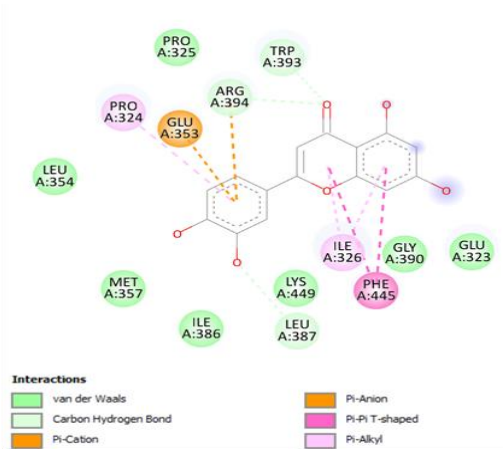
The docking study was conducted to assess the binding affinity of selected compounds with estrogen receptor involved in breast cancer regulation. PyRx was employed to perform the molecular docking simulations (Callil-Soares *et al*., 2023). The docking analysis revealed favorable binding interactions between Compounds and the selected receptors. The binding affinity was assessed based on the calculated binding energy and the formation of hydrogen bonds, hydrophobic interactions, and other molecular interactions.

The docking results indicated that Thymoquinone, Gallic acid, Apigenin, Luteolin, Patuletin and Myricetin forms stable complexes with Era. These interactions suggest the potential of these compounds as suitable drug candidate to modulate the activity of these receptors involved in cancer progression, breast cancer signaling, and inflammatory pathways.

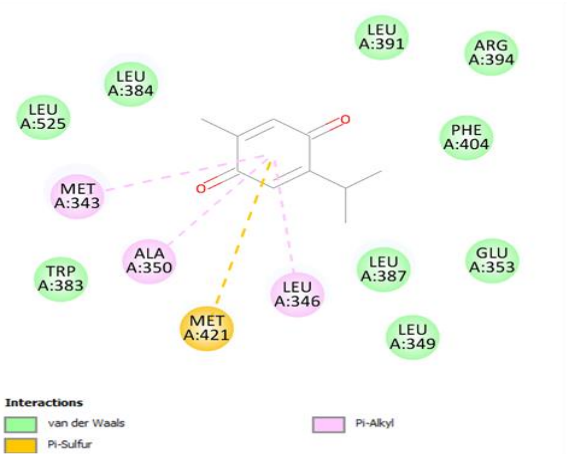
The binding affinities of the phytochemicals, along with their two-dimensional interactions and three-dimensional binding modes, are illustrated in Figure 1 and Figure 2.



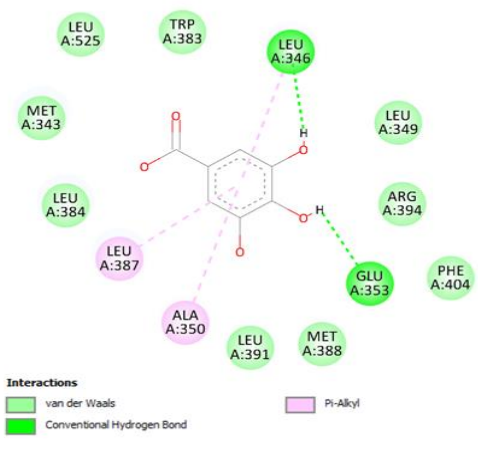
1-Apigenin



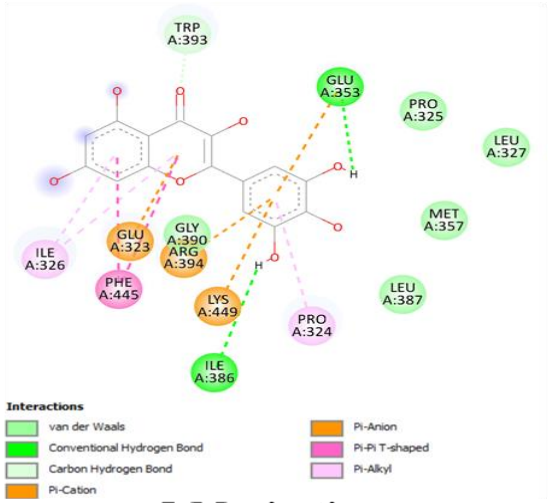
2-Luteolin



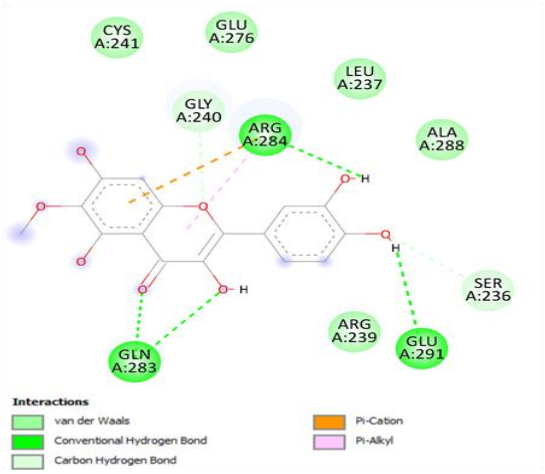
3-Thymoquinone



4-Gallic acid



5-Myricetin



6-Patuletin

Figure 1 Two dimensional representation of Thymoquinone, Gallic acid, Apigenin, Luteolin, Patuletin and Myricetin in the binding pocket of estrogen receptor, PDB 3ERT

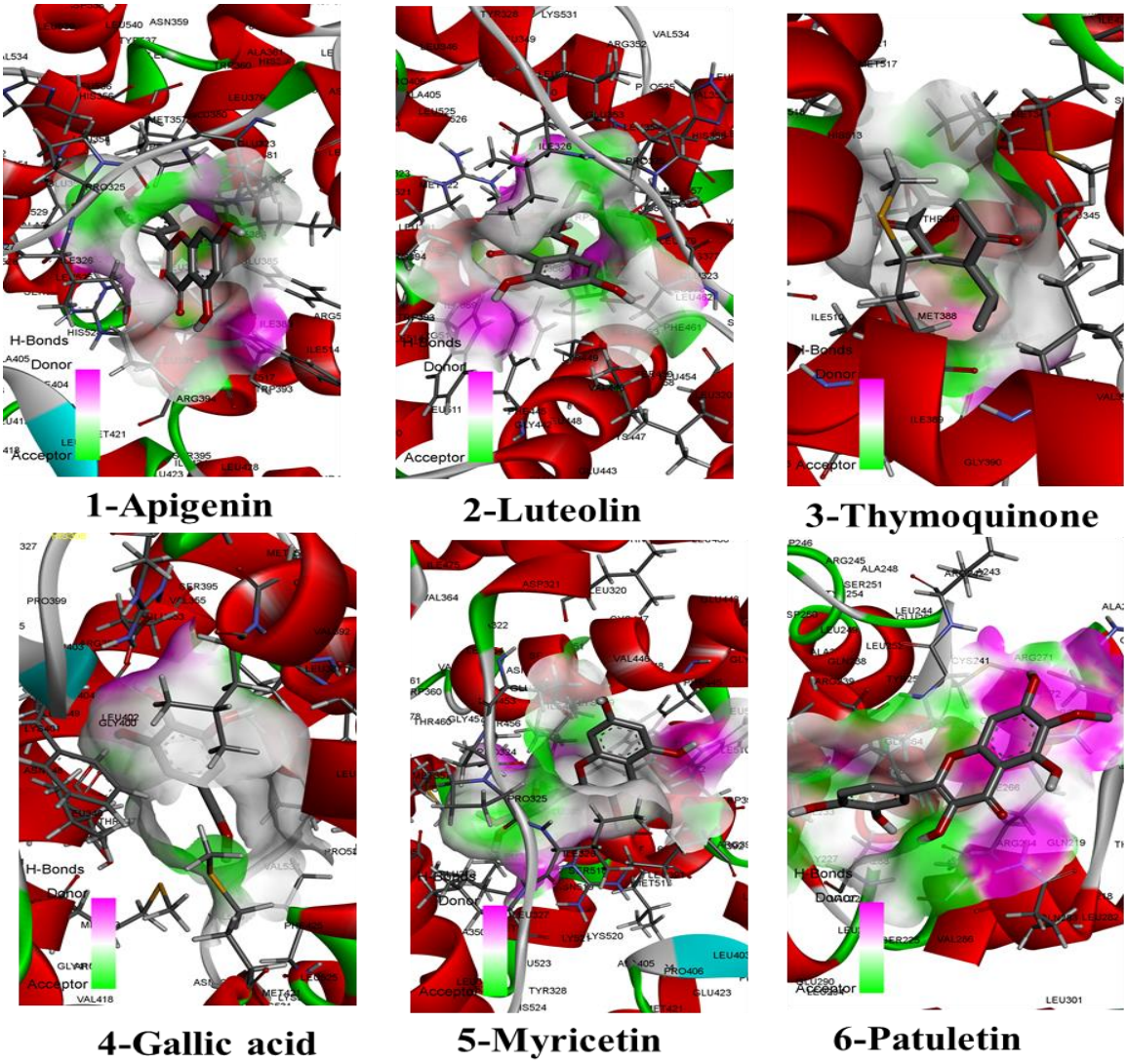


Figure 2 Docking pose of Thymoquinone, Gallic acid, Apigenin, Luteolin, Patuletin and Myricetin within the cavity of ERa

Discussion

The study utilized an integrated approach of molecular docking and virtual screening to explore the structural interactions of bioactive compounds from Nigella sativa with key molecular targets associated with breast cancer development. Herbal remedies like curcumin are widely preferred due to their minimal side effects and established therapeutic potential in cancer therapy[7]. [20]The network pharmacology approach helps elucidate the intricate interactions between drugs and their molecular targets, providing insights into their underlying mechanisms of action[20].The estrogen receptor (ER) was identified as the top-ranking target, suggesting its critical role in breast cancer treatment. Studies have shown that ER is expressed in around 75% of breast cancer cases. Targeting and modulating ER activity has significantly enhanced the survival rates of patients diagnosed with ER-positive breast cancer[21]

The druggability and ADMET properties of these compounds were assessed through in silico methods. Compounds displaying poor ADMET characteristics are typically filtered out and not advanced to clinical trials during the drug discovery process[22]. All selected compounds from screening fulfilled the Lipinski's Rule of Five as shown in table 2 and optimized ADMET profiles shown in table 3. Their human intestinal absorption (HIA), bioavailability, and ROCT substrate status indicated benefits such as lower efflux, enhanced renal clearance, and improved bioavailability, contributing to an optimal pharmacokinetic profile. Furthermore, a P-glycoprotein substrate model,

the P50 isoenzyme model associated with about 75% of drug metabolism via cytochrome clusters, confirmed the potential of both compounds as suitable drug candidates. Additionally, these compounds were shown to be non-inhibitors of CYPEC9 and CYP2D6 enzymes within this group. Thus, incorporating these bioactive compounds with favorable drug gable characteristics and an ideal ADMET profile in drug formulations may offer advantages over synthetic agents like acarbose, which is known for its metabolic instability[23]

The molecular docking analysis offered detailed insights into the interaction patterns and binding residues of these compounds at the active sites estrogen receptor shown in figure 1 and 2. This information helps to understand how these compounds engage with the target proteins. Research has shown that estrogen binds to estrogen receptors (ERs) and directly interacts with membrane receptors like IGFR, EGFR, and HER2, along with key signaling molecules. This activates major signaling cascades, including the MAPK and PI3K/AKT pathways, which in turn promote tumor cell proliferation, growth, and survival. Consequently, targeting the estrogen signaling pathway by either reducing estrogen production or inhibiting ER activity is considered an effective strategy for breast cancer treatment[24]

Conclusion

Given the devastating impact breast cancer has had over the years, there is an urgent need for effective therapeutic solutions. In our research, 60 phytochemicals from black cumin were initially screened based on pharmacokinetic properties, narrowing down to 9 promising compounds. These selected molecules were then subjected ADMET profiling and molecular docking against key breast cancer-related protein ERa, identifying the top 6 strongest binders. Further evaluation of bioavailability and toxicity suggested that Thymoquinone, Gallic acid, Apigenin, Luteolin, Patuletin and Myricetin are safe and effective against these targets. These findings have the potential to support the development of novel, phytochemical-based therapeutic strategies, offering valuable leads for further optimization in breast cancer drug design. Future research, including molecular dynamics simulations and in vivo studies, will be essential to validate these initial results and pave the way for new targeted therapies.

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