

# In-Silico Identification of Phytochemical Modulators Targeting Estrogen Receptor Alpha for Endometrial Carcinoma Therapy

Mustake Ahmed Robin<sup>1</sup>, Rubel Sarder<sup>2</sup>, Md. Sujon Ahmed<sup>3</sup>,  
Israt Jahan Ira<sup>4</sup>, T. S. Farah Mousumi<sup>5</sup>, Tanbirul Azim Maharaj<sup>6</sup>,  
Kazi Ashfak Ahmed Chowdhury<sup>7</sup>

<sup>1,2,3</sup>Department of Pharmacy, Mawlana Bhashani Science and Technology University, Tangail, Dhaka-1902

<sup>4</sup>Associate Professor, Department of Pharmacy, Mawlana Bhashani Science and Technology University, Tangail, Dhaka-1902

<sup>5</sup>Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342

<sup>6</sup>International Islamic University Chittagong, Chattogram-4318

<sup>7</sup>Associate Professor and Chairman, Department of Pharmacy, International Islamic University Chittagong, Chattogram-4318

## Abstract

There are major therapeutic problems connected with hormone receptor-positive cancer, which is driven by estrogen receptor alpha (ER $\alpha$ ). These challenges arise because of the resistance and side effects associated with conventional medications such as Tamoxifen. The purpose of this study is to identify novel phytochemical-based modulators that target the ER $\alpha$  ligand-binding domain (PDB ID: 3ERT) by utilizing an in-silico technique. Utilizing PyRx in conjunction with AutoDock Vina, a library consisting of 1,346 phytochemicals from the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database was screened for molecular docking. Among the leading contenders are luteoxanthin (-10.9 kcal/mol), 2,3-diphenylbenzofuran (-10.2 kcal/mol), alpha-carotene (-10.3 kcal/mol), and sesaminol (-9.7 kcal/mol). surpassed Tamoxifen (-7.0 kcal/mol) in terms of binding affinity, engaging important residues such as MET421, ASP451, LEU387, and ARG394 through hydrogen bonds and hydrophobic interactions, according to the findings of an analysis conducted with BIOVIA Discovery Studio. With the use of SwissADME and pkCSM, pharmacokinetic and toxicity profiles were evaluated, and the results showed that there was a high gastrointestinal absorption (more than 90 per cent), that Lipinski's Rule of Five was followed, and that there were minimal toxicity risks. To assess the stability of the binding process, 100-nanosecond molecular dynamics simulations were carried out using GROMACS. The results demonstrated stable relative mean square deviation (RMSD) values ranging from 0.25 to 0.29 nanometers. Additionally, consistent metrics were observed for RMSF, radius of gyration, hydrogen bonding, SASA, PSA, and MOLSA, thereby confirming the structural compatibility of the compounds with ER $\alpha$ . Based on these findings, the phytochemicals that have been found serve as promising leads for the development of selective ER $\alpha$  modulators that have the potential to increase both efficacy and safety in comparison to the medicines that are currently being utilized. Through the

integration of traditional phytomedicine with contemporary computational techniques, this study provides a robust pathway for drug development. In the future, efforts will be aimed towards experimental validation in order to advance these candidates into feasible treatments for ER $\alpha$ -positive cancer.

**Keywords:** Estrogen Receptor Alpha, Endometrial Carcinoma, Phytochemicals, Molecular Docking, Molecular Dynamics Simulation

## 1. Introduction

The most common gynecological cancer in developed nations is endometrial carcinoma, a malignant tumour that develops from the uterine lining. According to the American Cancer Society's predictions, there are over 65,000 new cases and about 12,000 fatalities in the US annually [1]. It is typically separated into two groups: Type I, which is endometrioid, and Type II, which is non-endometrioid and includes serous or clear cells. About 80% of cases are type I cancers, which are mostly estrogen-dependent and frequently linked to dysregulated activity or overexpression of ER $\alpha$  [2]. Increased ER $\alpha$ -mediated signaling leads to increased cell proliferation and decreased apoptosis, leading to Type I endometrial cancer development. Prolonged ER $\alpha$  activation occurs when estrogen is unopposed, whether from endogenous synthesis or external sources like hormone treatment [3]. Although type II endometrial carcinomas are less estrogen-dependent, they may still express ER $\alpha$ , but p53 mutations and aggressive tumor behavior are more directly linked to their development [4]. Late-stage endometrial cancer has below-20% survival rates, underlining its severity. Surgery, chemotherapy, radiation, and long-term care cost a lot, and the ailment affects many people [5]. These difficulties show the importance of new therapeutic strategies that enhance patient outcomes and lessen the cost burden on healthcare systems.

The nuclear receptor superfamily, which includes ligand-dependent transcription factors important in controlling a variety of physiological processes, includes estrogen receptor alpha (ER $\alpha$ ) as a crucial factor. The ESR1 gene encodes ER $\alpha$ , which is pivotal in mediating the effects of estrogen, a steroid hormone crucial for maintaining reproductive, metabolic, and skeletal equilibrium [6]. ER $\alpha$  exhibits significant expression in the endometrium, breast, and ovaries, playing a crucial role in regulating cell proliferation, differentiation, and apoptosis. Within the endometrium, it facilitates estrogen-driven growth throughout the proliferative phase, a mechanism regulated by progesterone receptor (PR) signaling that constrains ER $\alpha$  activity during the secretory phase. The transcriptional regulation of ER $\alpha$  is enhanced by the involvement of co-activators such as SRC-1 and CBP/p300, as well as co-repressors like NCoR and SMRT, which together ensure meticulous control over gene expression [7, 8]. To maintain endometrial homeostasis, ER $\alpha$  and PR signals must be in balance. Through PR, progesterone inhibits ER $\alpha$  by lowering the expression of ESR1 and directing the endometrium into a secretory, differentiated phase that facilitates implantation. ER $\alpha$  becomes too active when this equilibrium is upset, as in the case of unopposed estrogen exposure during anovulatory cycles or postmenopausal hormone therapy. This increases the risk of endometrial hyperplasia and eventual carcinogenesis [9]. In pharmaceutical research, *in silico* drug discovery has emerged as a potent strategy to overcome these obstacles. Scientists can forecast how tiny compounds will interact with targets like ER $\alpha$  by using computational techniques like molecular docking, virtual screening, and QSAR modeling [10]. A successful model for drug discovery is produced by combining *in silico* and *in vivo* techniques. To focus experimental trials on the most promising candidates and expedite the development process,

computational techniques first screen and select compounds [11]. Pharmacokinetic and toxicity assessments are crucial in early drug development, providing insights into a compound's ADMET characteristics. In preclinical and clinical research, bioavailability, therapeutic window, and safety profile predict a molecule's success. The orientation and binding strength of small molecules within the ER $\alpha$  ligand-binding domain (LBD) are estimated using molecular docking. High-affinity and specificity molecules can be found using docking by mimicking interactions with important residues [12]. A dynamic view of ER $\alpha$ -ligand interactions is provided by molecular dynamics (MD) simulations, which show binding stability and conformational changes throughout time. MD studies assist in identifying mechanisms of ligand-induced conformational alterations, such as H12 repositioning, by modeling ER $\alpha$  complexes with possible inhibitors [13]. This study assesses the impact of solvent effects, protein flexibility, and co-regulator interactions, thereby enhancing the understanding of ER $\alpha$  antagonism. Molecular dynamics (MD) simulation tools, including GROMACS and AMBER, are extensively utilized, providing high-resolution insights into ligand-receptor dynamics [14]. Pharmacokinetics and possible toxicity are estimated using machine learning models, whereas binding affinities and important interactions within the receptor's ligand-binding region are predicted via molecular docking. Large chemical libraries can be quickly screened using these techniques, which outperform conventional experimental techniques and save time and money.

The current study seeks to identify new therapeutic agents that target the ligand-binding domain of human estrogen receptor alpha (ER $\alpha$ ), which plays a crucial role in the development and progression of hormone receptor-positive endometrial carcinomas. This study utilizes an integrative computational approach that merges structure-based virtual screening, molecular docking, and phytochemical analysis to identify natural compounds exhibiting high affinity for ER $\alpha$ . The aim is to identify potential lead molecules that may function as selective estrogen receptor modulators (SERMs) or inhibitors, potentially providing improved pharmacological profiles relative to current treatments like tamoxifen.

## 2. Materials and Methods

### 2.1. Preparation of The Target Protein

The three-dimensional structure of the human estrogen receptor alpha (ER $\alpha$ ) ligand-binding domain was retrieved from the RCSB Protein Data Bank under the PDB ID 3ERT. This domain, which plays a key role in regulating gene expression upon ligand binding, was chosen due to its well-defined conformation in complex with 4-hydroxytamoxifen, making it a robust structural template for computational analyses. The deposited structure consists of a single protein chain with a molecular weight of approximately 30.24 kDa, comprising 261 residues (247 of which are modelled) and 2,070 [15]. These characteristics render the structure well-suited for accurate structure-based virtual screening and detailed interaction analysis. Initial preparation was carried out using PyRx, during which non-essential components such as the co-crystallized ligand, water molecules, heteroatoms, and other extraneous structural elements were meticulously removed. This step ensured that only the isolated ligand-binding domain of the receptor, specifically its binding pocket, was preserved for subsequent computational simulations. To refine the geometry and enhance structural stability, energy minimization was performed using Swiss-PDB Viewer (SPDBV). This process alleviated unfavorable steric clashes and optimized bond angles and torsional parameters, yielding a low-energy conformation that is well-suited for subsequent molecular docking studies [16]. The final refined structure was subsequently converted into PDBQT format to ensure compatibility with AutoDock Vina within the PyRx platform. This optimized receptor model was then

used as a prepared target for virtual screening and molecular docking of selected compounds, thereby supporting reliable, consistent, and reproducible computational analyses.

## 2.2. Compounds Retrieval and Preparation

Phytochemicals derived from medicinal plants constitute a rich and largely underexplored reservoir of structurally diverse bioactive molecules, offering substantial promise for the discovery of novel receptor modulators. In this study, a carefully curated library of 1,480 plant-derived compounds was compiled from the Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT 2.0) database (<https://cb.imsc.res.in/imppat/>). This comprehensive resource provides detailed data on phytochemical structures, traditional therapeutic uses, and botanical origins, drawing from established systems of traditional medicine—including Ayurveda, Siddha, and Unani thereby bridging ethnopharmacological knowledge with modern drug discovery approaches [17]. Each compound in the dataset was acquired in both Structure Data File (SDF) and canonical SMILES (Simplified Molecular Input Line Entry System) formats to ensure broad compatibility with diverse cheminformatics tools and molecular docking platforms. These standardized representations enabled efficient processing, accurate 3D structure generation, and reliable ligand preparation during virtual screening. For benchmarking purposes, the control ligand 4-hydroxytamoxifen—a well-characterized antagonist of the human estrogen receptor alpha—was also retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in identical SDF and SMILES formats. This established reference compound provided a critical baseline for assessing the relative binding affinities and interaction patterns of the phytochemical candidates. The fully curated molecular library, comprising 1,480 plant-derived compounds from the IMPPAT 2.0 database alongside the reference ligand, was subsequently prepared for structure-based virtual screening against the ligand-binding domain of ER $\alpha$  (PDB ID: 3ERT) to systematically evaluate their potential as novel receptor modulators [18].

## 2.3. Active Site Identification

The active site of a protein is a structurally well-defined region composed of key amino acid residues that are essential for the recognition and binding of small-molecule ligands. These sites are functionally crucial, as they facilitate molecular interactions through a combination of non-covalent forces, including hydrogen bonds, hydrophobic contacts, electrostatic interactions, and van der Waals forces that together confer binding specificity and complex stability. In this study, the ligand-binding site of the human estrogen receptor alpha (ER $\alpha$ ) ligand-binding domain (PDB ID:3ERT) was systematically identified and characterized to define the most suitable pocket for molecular docking. To achieve this, the CASTp 3.0 server (Computed Atlas of Surface Topography of Proteins) was utilized. CASTp provides high-precision detection and delineation of surface pockets and internal cavities by analyzing geometric and topological features of the protein structure, thereby offering detailed insights into ligand-accessible regions that are likely to serve as functional binding sites. This computational mapping was instrumental in guiding subsequent docking simulations with phytochemical candidates [19]. Among the surface cavities identified by CASTp 3.0, the largest and most topologically accessible pocket was selected for subsequent docking studies. This pocket encompasses several functionally critical residues MET421, ASP451, LEU387, and ARG394, which are well-documented for their central role in ligand recognition and binding within the estrogen receptor alpha ligand-binding domain. The spatial arrangement and interaction capabilities of these residues were meticulously evaluated to confirm their involvement in forming a biologically relevant binding interface. To further validate the structural integrity and geometry of the selected pocket, detailed visual inspection and analysis were conducted using BIOVIA

Discovery Studio Visualizer. This step enabled a comprehensive characterization of the pocket's architecture, including the identification of key interaction hotspots and potential hydrogen-bonding or hydrophobic regions. Such validation was essential to ensure that subsequent molecular docking simulations would be both structurally accurate and biologically meaningful, thereby enhancing the reliability of the virtual screening outcomes [20]. This method improves the prediction accuracy of docking simulations by fully describing the receptor's binding environment, providing a strong basis for finding ligands with high affinity and specificity for ER $\alpha$ 's ligand-binding region.

#### **2.4.Molecular Docking**

A crucial computational method used in structure-based drug development to forecast how tiny compounds would interact with target proteins is molecular docking. The binding affinity, orientation, and important intermolecular interactions between ligands and receptor sites are estimated using this method. PyRx, which integrates the AutoDock Vina engine to enable quick and precise assessment of ligand binding to the receptor, was used in the current study's docking simulations [21]. The ligand-binding domain of the human estrogen receptor alpha (PDB ID: 3ERT) was the target for docking. A wide range of 1,480 phytochemical compounds selected from the IMPPAT 2.0 database were screened to find molecules that might successfully interact with the active site of the receptor. The grid box dimensions were roughly 50.45 Å  $\times$  58.26 Å  $\times$  55.93 Å along the x, y, and z axes, respectively, and the center coordinates were set at x = 22.5215, y = 5.3825, and z = 21.8343 to ensure accurate docking within the biologically relevant region. During the docking process, ligands were able to explore the crucial interaction space because this arrangement successfully covered the main ligand-binding pocket. Strong anticipated binding affinity and stable interactions were indicated by ligands with the most favorable (i.e., lowest) binding energies, which were screened as possible options. The molecular interaction features of the obtained docked complexes were further examined using BIOVIA Discovery Studio Visualizer. These included  $\pi$ - $\pi$  stacking interactions, hydrogen bonds, and hydrophobic contacts all of which are essential for maintaining ligand-receptor complex stability. The binding mechanisms of the top-ranking compounds were revealed by this thorough interaction study, which also found important amino acid residues that greatly influence the stability and specificity of binding. The identification of prospective modulators of the estrogen receptor alpha ligand-binding domain was finally advanced by these insights, which guided the logical selection of lead compounds for ensuing pharmacokinetic assessments and molecular dynamics simulations.

#### **2.5.Pharmacokinetics Properties and Toxicity Prediction**

A key factor in assessing the effectiveness and safety profile of possible medication candidates is pharmacokinetics, which includes the processes of absorption, distribution, metabolism, and excretion (ADME). A critical stage in the early screening of small molecules is the in-silico evaluation of these parameters, which enables researchers to analyze the drug-like properties of compounds and forecast how they would behave in biological systems [22]. The physicochemical characteristics, bioavailability, and medicinal chemistry features of a few phytochemical modulators that target the ligand-binding domain of the human estrogen receptor alpha (PDB ID: 3ERT) were thoroughly examined in this study using the SwissADME tool (<http://www.swissadme.ch/index.php>). The predictive capabilities of SwissADME allow for the effective assessment of crucial elements, including solubility, gastrointestinal absorption, and compliance with accepted drug-likeness guidelines [23, 24]. Toxicity profiling is similarly essential for early detection of substances with potentially dangerous effects, such as hepatotoxicity, cardiotoxicity, or off-target toxicities, and it complements this study. In order to do this,

certain pharmacokinetic characteristics and toxicity risks were predicted using the pkCSM web server (<https://biosig.lab.uq.edu.au/pkcsm/prediction>). pkCSM predicts important endpoints such as metabolic stability, clearance rates, possible cardiotoxicity (e.g., hERG channel blockage), hepatotoxicity, and other pertinent toxicological issues by utilizing graph-based signatures in conjunction with machine learning techniques. By incorporating these computational platforms into the workflow, a reliable and methodical selection of lead phytochemicals is ensured, matching favorable safety profiles with appropriate pharmacological activity [25]. In the search for efficient modulators of the estrogen receptor alpha ligand-binding domain, our method expedites the prioritizing of potential candidates for additional experimental validation.

## 2.6. Molecular Dynamics (MD) Simulation

The binding stability and dynamic behaviour of several phytochemical candidates, including luteoxanthin (IMPHY002029, docking score: -10.9), 2,3-Diphenylbenzofuran (IMPHY003017, docking score: -10.2), alpha-Carotene (IMPHY011609, -10.3), and sesaminol (IMPHY012971, docking score: -9.7) in the ligand-binding domain of the human estrogen receptor alpha. GROMACS 2023.3 on a Linux Ubuntu 22.04.2 LTS system was used to run the simulations, and the CHARMM27 all-atom force field was used for precise molecular modeling. A. To provide smooth interaction with the CHARMM force field, ligand topology and parameters were produced using SwissParam. A solvent buffer zone of 10 Å was maintained around each protein-ligand interaction by submerging it in an orthorhombic simulation box filled with TIP3P water molecules. The systems were neutralized by adding 0.15 M NaCl to replicate physiological ionic conditions. In order to resolve unfavorable contacts, the simulation protocol started with 5000 steps of energy minimization. Next, the SHAKE algorithm constraints were applied to hydrogen bonds in order to preserve bond stiffness. The system was gradually heated to 310 K to establish temperature equilibration. This was followed by equilibration phases in the NVT (constant Number, Volume, Temperature) and NPT (constant Number, Pressure, Temperature) ensembles, each lasting 100 picoseconds at 310 K and 1 atm. Throughout the production run, the Berendsen barostat controlled pressure and the V-rescale thermostat-controlled temperature. To evaluate the conformational stability and flexibility of the complexes, thorough analyses of structural parameters such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), radius of gyration (Rg), and hydrogen bond occupancy were carried out during the 100 ns production simulation. These findings supported the phytochemicals' potential as candidate molecules for modifying the estrogen receptor alpha ligand-binding domain by offering crucial insights into the binding kinetics and possible inhibitory effects of the compounds in comparison to the control.

## 3. Result

### 3.1. Phytochemical Retrieval and Preparation

The main source for compound selection was the IMPPAT 2.0 database, a carefully curated collection of bioactive phytochemicals from Indian medicinal plants. Tamoxifen, which was obtained from the PubChem database, was used as the standard reference ligand for the virtual screening of 1,346 phytochemical compounds against the human estrogen receptor alpha ligand-binding domain. After all molecules were first acquired in SDF format, their three-dimensional conformations were optimized by energy minimization. Open Babel, which is incorporated into the PyRx platform, was then used to convert the minimized ligands to PDBQT format. This preprocessing procedure improved the precision

and dependability of the molecular docking analyses while guaranteeing complete compatibility with AutoDock Vina.


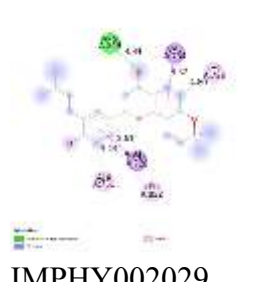
### 3.2. Active Sites of Target Proteins and Receptor Grid Configuration

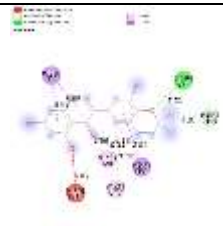
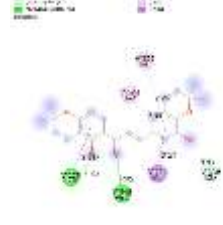

Certain amino acid residues found in proteins create active sites that allow ligand binding via non-covalent interactions such as van der Waals forces, hydrophobic contacts, and hydrogen bonds. Four crucial residues, MET421, ASP451, LEU387, and ARG394 were shown to be essential for ligand recognition and interaction in the ligand-binding domain of the human estrogen receptor alpha examined in this work. The docking grid was properly centered around these crucial residues in order to reliably assess binding. By ensuring that docking simulations accurately recorded pertinent ligand receptor interactions, this focused grid layout improved the accuracy of ligand location and the identification of possible inhibitors inside the active region.

### 3.3. Molecular Docking Analysis

An essential in-silico method for forecasting the best binding orientations and interaction strengths between target proteins and putative bioactive compounds is molecular docking. In this investigation, AutoDock Vina was used to screen 1,346 phytochemicals from the IMPPAT 2.0 database against the ligand-binding domain of estrogen receptor alpha (ER $\alpha$ , PDB ID: 3ERT). The resulting binding affinities varied between -1.1 and -11.3 kcal/mol. Compared to the reference medication Tamoxifen, which had a docking score of -7.0 kcal/mol, a number of drugs showed greater binding (Table 1). Significant inhibitory potential was shown by the strong anticipated interactions these top-scoring phytochemicals established with important active-site residues. Promising candidates were then given priority for pharmacokinetic and toxicology analyses, highlighting their potential as ER $\alpha$  modulators for upcoming treatment advancements.

**Table 1: Molecular Docking Score of the Selected Compounds**

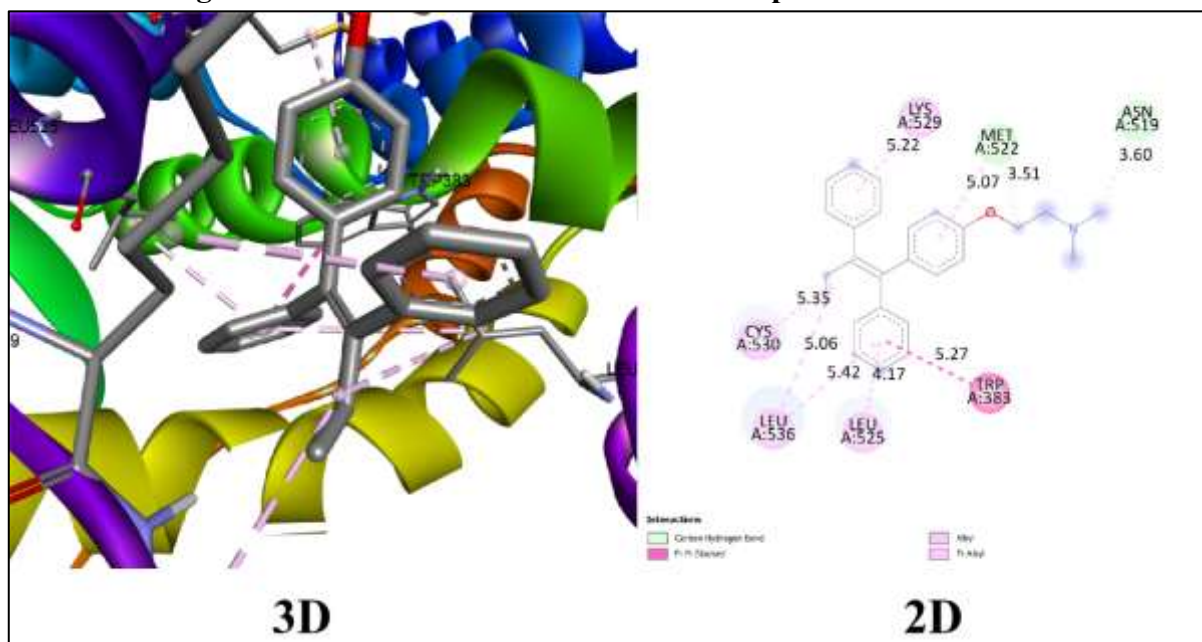
PDB ID of Protein	Chemical Structure of Compounds	Binding Affinity (kcal/mol)	Amino Acid Interaction	
			Hydrogen Bonds	Hydrophobic Bonds
3ERT	 2733526	-7.0 kj/mol	MET 522, ASN 519	LYS 529, TRP 383, LEU 525, LEU 536, CYS 530
3ERT	 IMPHY002029	-10.9 kj/mol	GLU 419	LEU 391, LEU 387, ALA 350, LEU 384, PRO 324

3ERT	 IMPHY003017	-10.2 kj/mol	LEU 384	ILE 424, LEU 525, LEU 346, LEU 387, LEU 391, LEU 349
3ERT	 IMPHY011609	-10.3 kj/mol	LEU 347	LEU 525, ALA 350, LEU 391, ILE 326, MET 357
3ERT	 IMPHY012971	-9.7 kj/mol	LEU 525	ALA 350, PHE 445, TRP 393, ILE 326

### 3.4. Protein-ligand Interactions

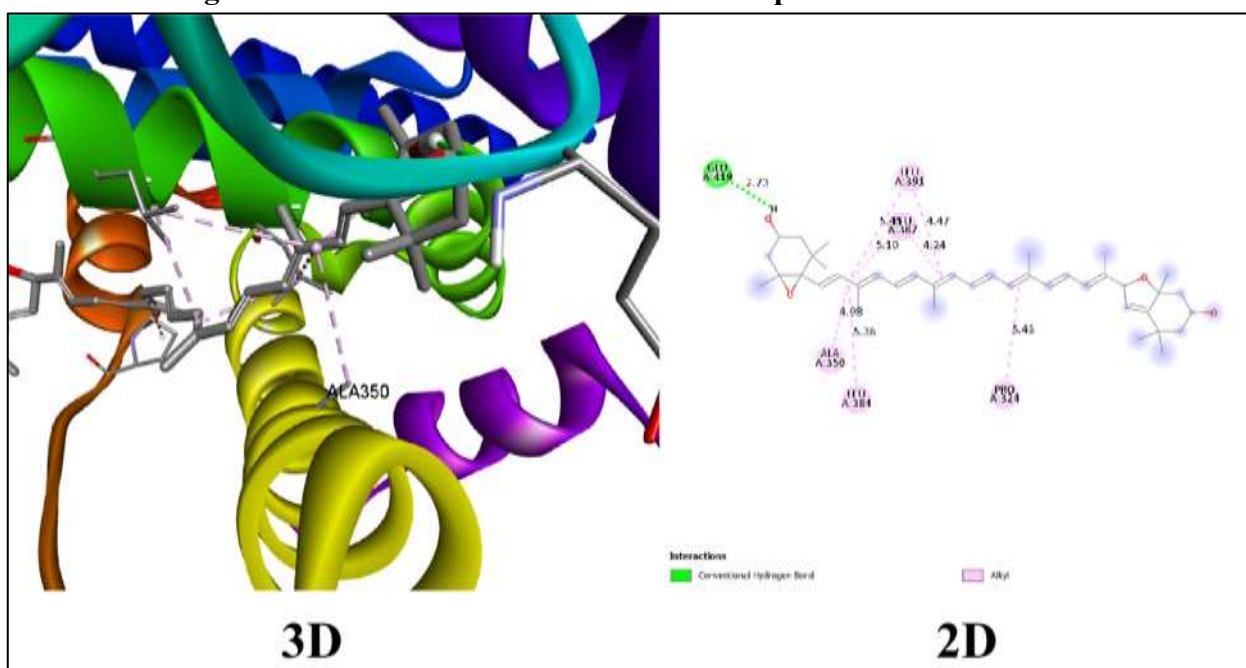
To clarify the binding behavior of particular phytochemical candidates inside the ligand-binding domain of the estrogen receptor, thorough interaction investigations were carried out utilizing BIOVIA Discovery Studio Visualizer. This assessment made it possible to see important chemical interactions in great detail, offering insights into the structural and functional underpinnings of ligand-receptor binding [26]. The stability and specificity of ligand binding were supported by the interaction studies, which showed a variety of hydrophobic and hydrogen-bonding interactions with important active-site residues. Tamoxifen (CID: 2733526), the reference drug, demonstrated a binding affinity of  $-7.0$  kcal/mol and generated moderately hydrophobic interactions, especially with residues MET421 and LEU387. The relative binding performance of the phytochemical candidates was evaluated using these interaction patterns as a standard. (Figure 1)

**Figure 1: 2D And 3D Interaction of the Compound CID: 2733526**



Luteoxanthin (IMPHY00209) showed a significantly higher binding affinity of  $-10.9$  kcal/mol. In addition to hydrophobic interactions with MET421 and LEU387, its interaction profile includes hydrogen bonds with important residues ASP451 and ARG394. Its strong potential as a promising estrogen receptor inhibitor is highlighted by this broad and clearly defined interaction network. (Figure 2)

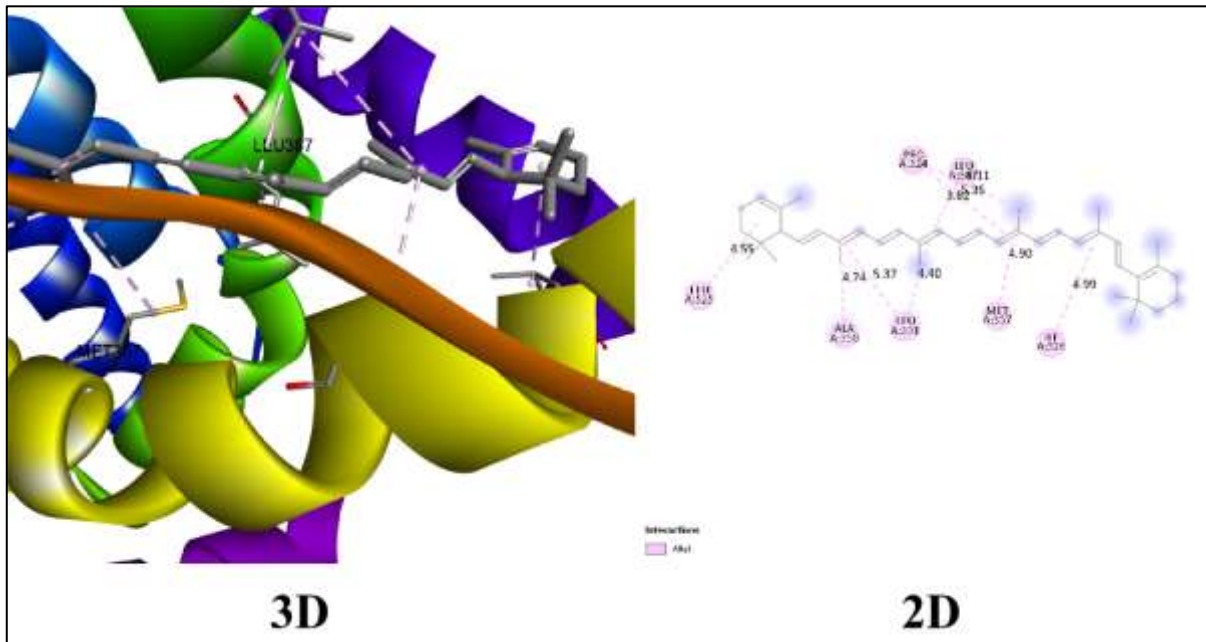
**Figure 2: 2D and 3D Interaction of the Compound IMPHY00209**



IMPHY011609 ( $\alpha$ -Carotene) showed good interactions with MET421, LEU387, and ARG394 through hydrophobic contacts and van der Waals forces, with a binding affinity of  $-10.3$  kcal/mol. Its potential

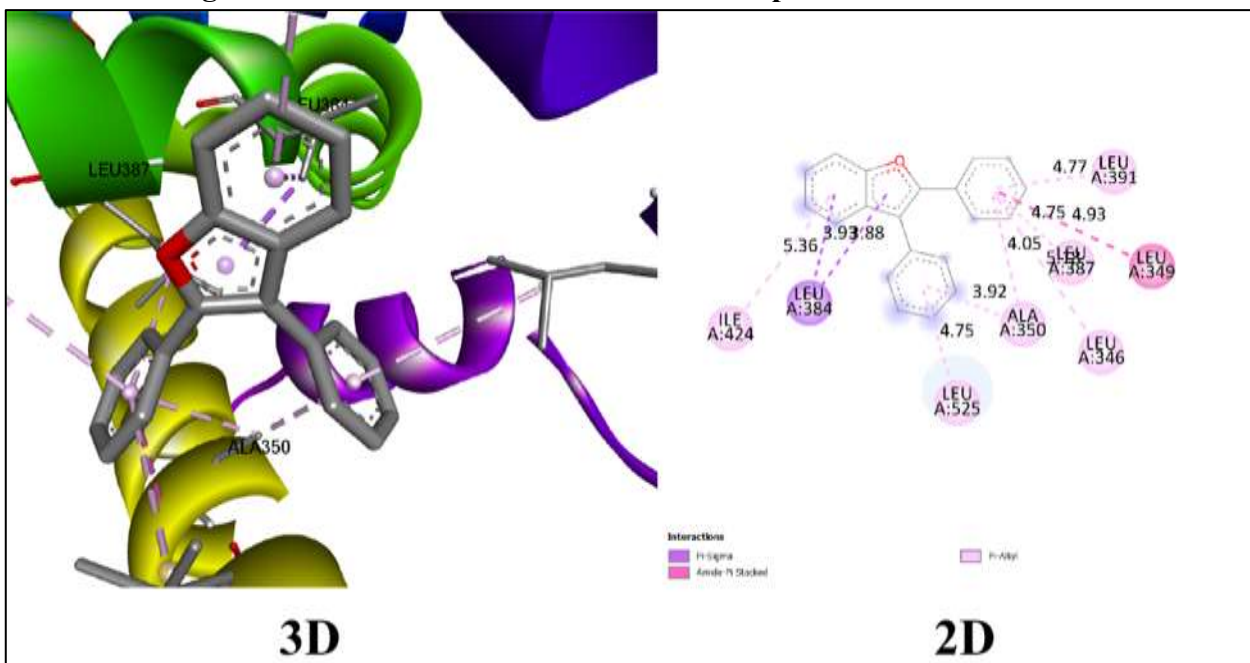
as a potent ER $\alpha$ -binding candidate is further supported by its extended hydrocarbon structure, which permits a well-fitting accommodation within the receptor's binding cavity. (Figure 3)

**Figure 3: 2D and 3D Interaction of the Compound IMPHY011609**



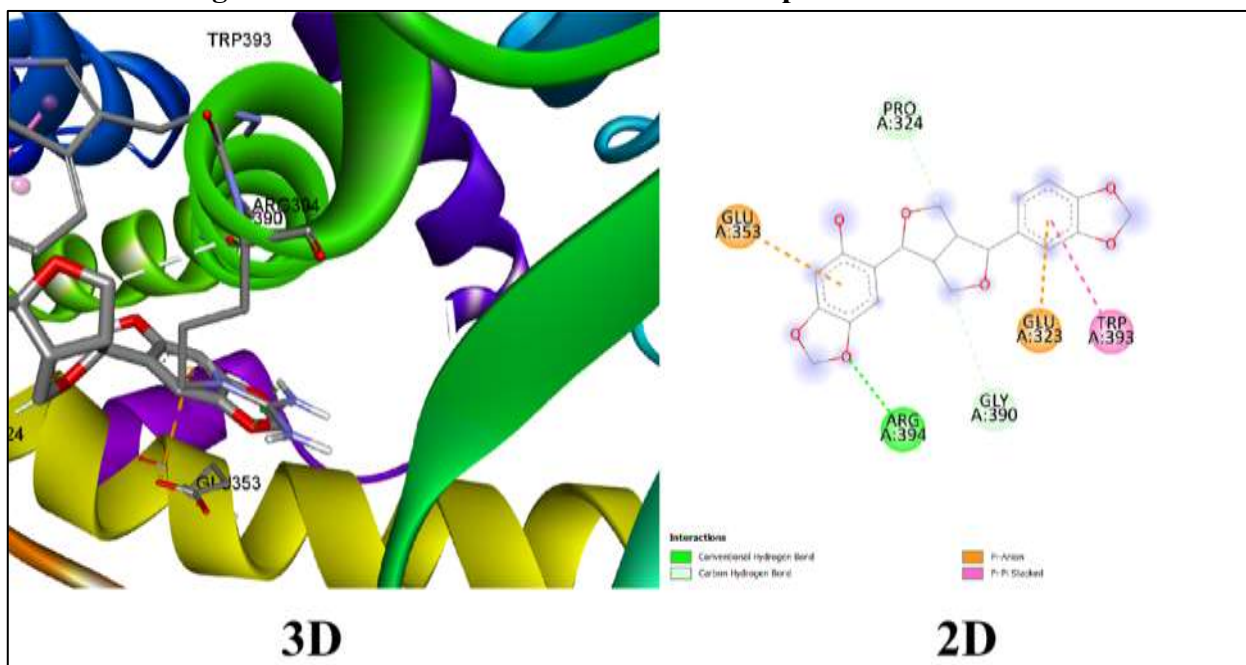
With a docking score of  $-10.2$  kcal/mol, IMPHY003017 (2,3-Diphenylbenzofuran) formed hydrogen bonds with ASP451 and nearby polar residues. Furthermore, a stable and highly specific binding orientation within the receptor's active region was facilitated by  $\pi$ - $\pi$  stacking and hydrophobic interactions with ARG394 and MET421. (Figure 4)

**Figure 4: 2D and 3D Interaction of the Compound IMPHY003017**



With a docking energy of  $-9.7$  kcal/mol, IMPHY012971 (Sesaminol) formed many hydrogen bonds with ARG394 and ASP451 in addition to hydrophobic contacts with LEU387 and MET421. Its potential as a potent and well-targeted ligand candidate for estrogen receptor regulation is highlighted by this harmonious mix of polar and nonpolar interactions. (Figure 5)

**Figure 5: 2D and 3D Interaction of the Compound IMPHY012971**



When taken as a whole, these interaction profiles show that the chosen phytochemicals had better binding potential than the control. Their progression to additional pharmacological investigation as prospective receptor modulators is highly supported by their capacity to generate numerous stabilizing contacts within the active region of the estrogen receptor.

### 3.5. Pharmacokinetics Properties

SwissADME and pkCSM were used to assess the pharmacokinetics, drug-likeness, and oral bioavailability of the top performing phytochemicals (Luteoxanthin, 2,3-Diphenylbenzofuran,  $\alpha$ -Carotene, and Sesaminol), with Tamoxifen serving as a reference. With good Caco-2 permeability, high gastrointestinal absorption ( $>90\%$ ), and compliance with Lipinski's Rule of Five, all compounds showed great promise as oral active, drug-like estrogen receptor modulators. The majority of compounds showed non-toxic profiles with no significant safety concerns, according to toxicity assessments utilizing pkCSM, including hepatotoxicity, AMES mutagenicity, hERG inhibition, and acute oral toxicity. When minor alarms are present, they support an overall favorable safety margin for early-stage drug development and call for additional preclinical research [27, 28]. The phytochemicals' potential as lead estrogen receptor modulators for additional preclinical research was supported by their higher binding and advantageous ADMET profiles in comparison to tamoxifen. (Table2)

**Table 2: ADME Properties of the Selected Compounds**

Property	Model Name	Unit	IMPPAT				PubChem CID
			IMPHY002029	IMPHY003017	IMPHY011609	IMPHY012971	2733526
Absorption	Caco2 permeability	Numeric (log Papp in 10-6 cm/s)	1.041	0.134	0.749	0.872	0.466
	Intestinal absorption (human)	Numeric (% Absorbed)	94.588	75.324	72.002	80.201	62.719
Distribution	VDss (human)	Numeric (log L/kg)	0.635	-0.977	0.026	-0.128	-0.651
Metabolism	CYP2D6 substrate	Categorical (Yes/No)	No	No	No	No	No
	CYP3A4 substrate	Categorical (Yes/No)	Yes	Yes	No	No	No
	CYP2D6 inhibitor	Categorical (Yes/No)	No	No	No	No	No
	CYP3A4 inhibitor	Categorical (Yes/No)	Yes	Yes	No	No	No
Excretion	Total Clearance	Numeric (log ml/min/kg)	0.018	0.014	0.46	0.312	-0.104
Toxicity	Oral Rat Acute Toxicity (LD50)	Numeric (mol/kg)	2.934	2.389	3.75	2.816	2.682
	AMES toxicity	Categorical (Yes/No)	No	No	No	No	No
	Hepatotoxicity	Categorical	No	No	No	No	Yes

		(Yes/No)					
	Minnow toxicity	Numeric (log mM)	-0.63	0.711	2.773	1.056	2.121

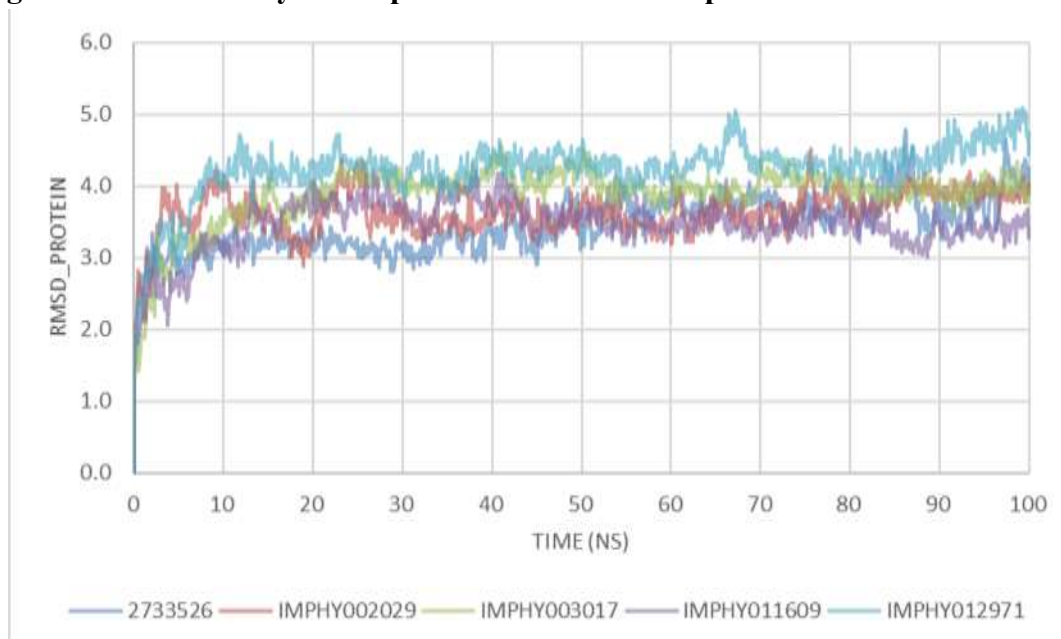
### 3.6. Molecular Dynamics (MD) Simulation Analysis

The structural stability and interaction dynamics of the top four phytochemicals, Luteoxanthin, 2,3-Diphenylbenzofuran,  $\alpha$ -Carotene, and Sesaminol, as well as Tamoxifen inside the ligand-binding region of the human estrogen receptor, were evaluated using molecular dynamics (MD) simulations. To assess atomic-level fluctuations, conformational changes, and interaction durability under near-physiological settings, each complex was simulated for 100 ns. For the phytochemical complexes, analyses of RMSD, RMSF, radius of gyration, and hydrogen-bonding patterns showed stable binding and preserved structural integrity. These findings support their continued experimental validation in drug discovery and offer compelling computational evidence of their potential as potent estrogen receptor modulators.

#### 3.6.1. RMSD Analysis

With average RMSD values of (0.25–0.29) nm, RMSD analysis from 100 ns MD simulations showed that the estrogen receptor complexes with luteoxanthin, 2,3-diphenylbenzofuran,  $\alpha$ -Carotene, and sesaminol remained stable, showing consistent structural integrity. The Tamoxifen complex, on the other hand, showed mild variations, especially in the 30–60 ns range. These results emphasize the potential of the chosen phytochemicals as potent therapeutic agents by indicating that they generate more stable and long-lasting connections with the receptor. (Figure 6)

**Figure 6: RMSD Analysis Graph of the Selected Compounds and the Control Drug**

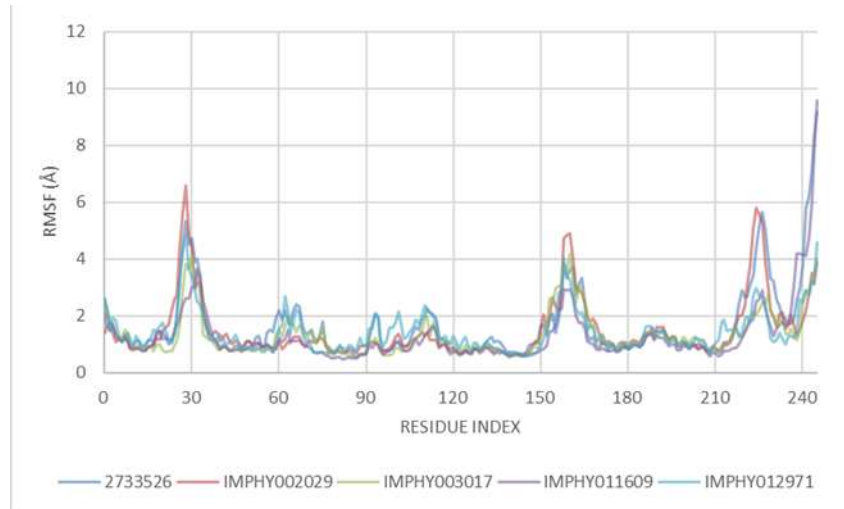


#### 3.6.2. RMSF Analysis

Receptor complexes containing luteoxanthin, 2,3-diphenylbenzofuran,  $\alpha$ -Carotene, and sesaminol showed minimal residue variations (0.10–0.16 nm) at important binding site residues, indicating robust local interactions, according to RMSF analysis. Tamoxifen, on the other hand, showed greater variations

(up to 0.35 nm) in some loops and helices, indicating decreased local stability. These findings demonstrate the phytochemicals' ability to stabilize the estrogen receptor binding pocket. (Figure 7).

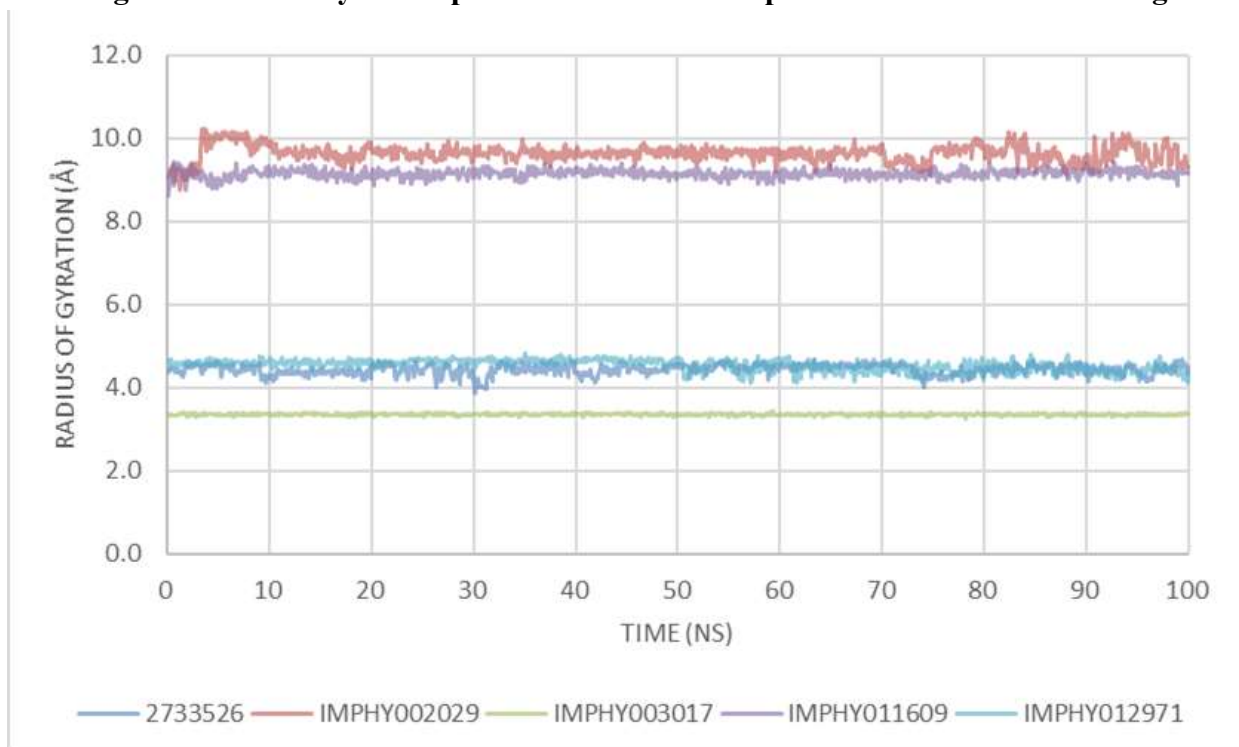
**Figure 7: RMSF Analysis Graph of the Selected Compounds and the Control Drug**



### 3.6.3. Rg (Radius of Gyration) Analysis

Estrogen receptor complexes with luteoxanthin, 2,3-diphenylbenzofuran,  $\alpha$ -Carotene, and sesaminol maintained steady Rg values of (2.31–2.36) nm over 100 ns, indicating intact protein compactness and structural integrity, according to radius of gyration (Rg) studies. The Tamoxifen-bound complex, on the other hand, showed slight oscillations, indicating slight structural relaxation. These findings highlight the phytochemicals' capacity to preserve the original fold of the receptor during binding. (Figure 8)

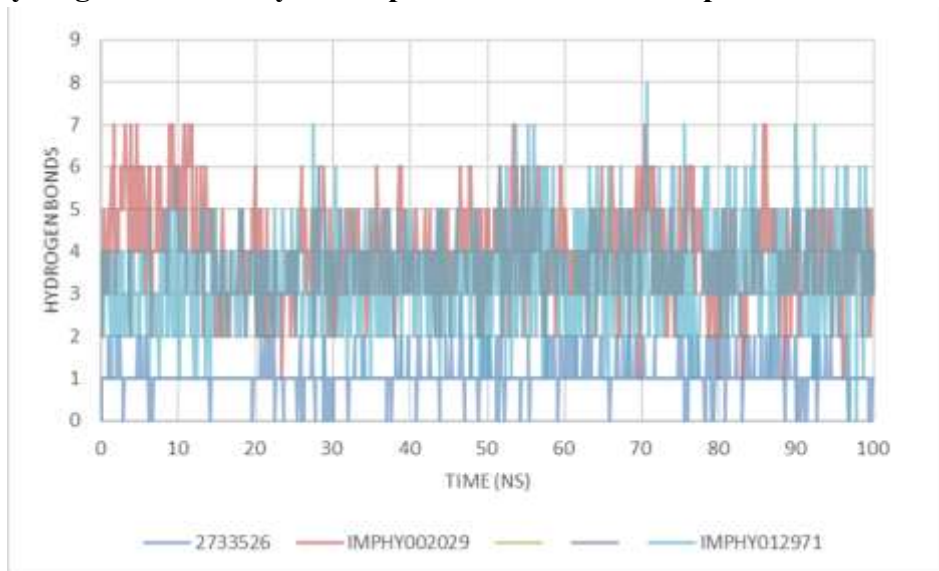
**Figure 8: RG Analysis Graph of the Selected Compounds and the Control Drug**



### 3.6.4. Analysis of Hydrogen Bonds

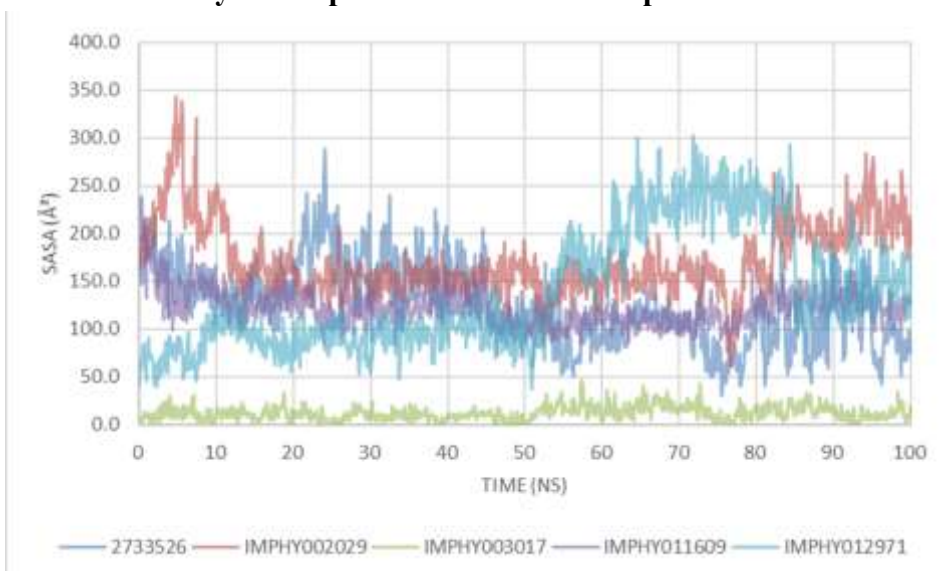
Luteoxanthin,  $\alpha$ -carotene, and sesaminol consistently interacted with important residues in the estrogen receptor binding domain, according to hydrogen bond studies conducted during 100 ns MD simulations. Interestingly, 2,3-diphenylbenzofuran demonstrated improved binding stability over time by exhibiting an increase in hydrogen bonds. Tamoxifen, on the other hand, created fewer and more erratic hydrogen bonds, demonstrating the phytochemical ligands' higher contact durability (Figure 9).

**Figure 9: Hydrogen Bond Analysis Graph of the Selected Compounds and the Control Drug**



Phytochemical-bound estrogen receptor complexes maintained stable surface areas (28,200–29,800 Å<sup>2</sup>), indicating continuous ligand accommodation, according to SASA analysis conducted across 100 ns MD simulations. The Tamoxifen complex, on the other hand, showed more variations, suggesting less stable binding. These findings support the phytochemicals' efficient and long-lasting interaction with the binding region of the receptor (Figure 10).

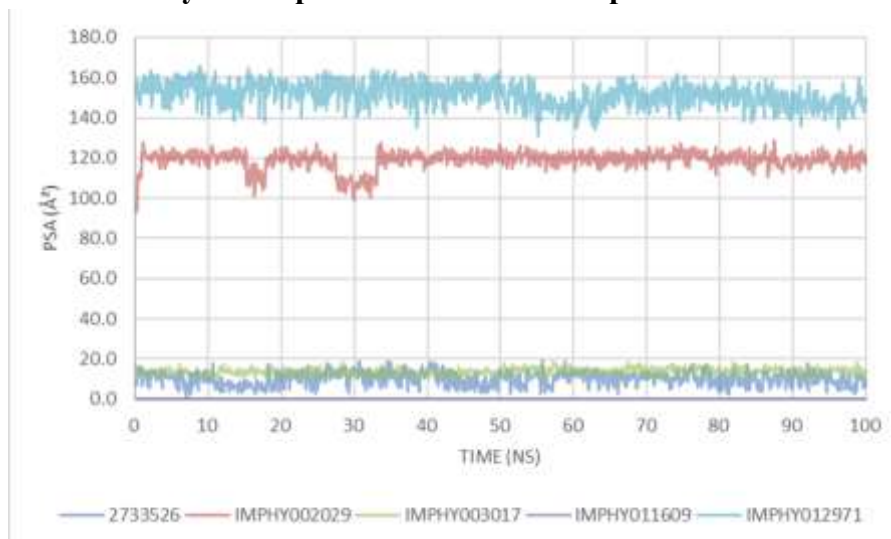
**Figure 10: SASA Analysis Graph of the Selected Compounds and the Control Drug**



### 3.6.5. PSA Analysis

The four phytochemicals showed consistent results throughout the simulation according to polar surface area (PSA) analysis, with 2,3-diphenylbenzofuran and sesaminol showing the lowest PSA, indicating favorable absorption and permeability. Tamoxifen's dynamic binding nature was reflected in its moderate PSA variations. The phytochemicals' drug-like potential is supported by their consistent PSA profiles (Figure 11).

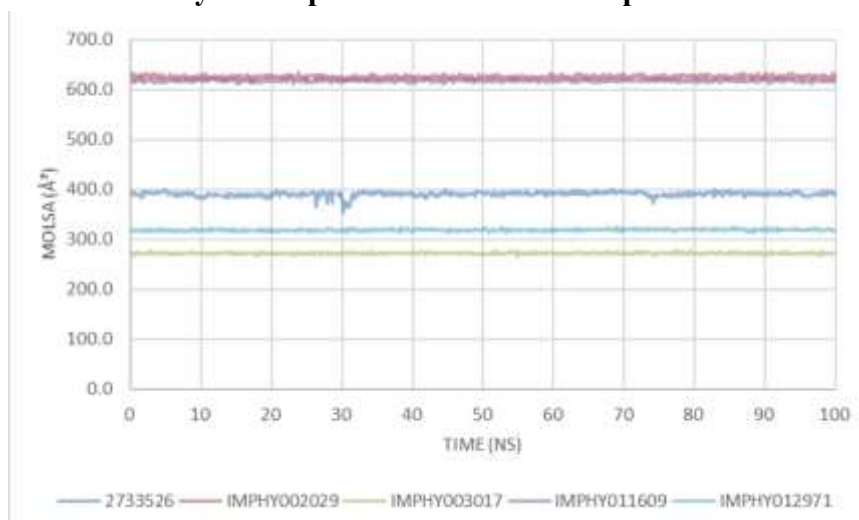
**Figure 11: PSA Analysis Graph of the Selected Compounds and the Control Drug**



### 3.6.6. MOLSA Analysis

All phytochemicals maintained steady ligand-exposed surface areas during the 100 ns simulation, according to MOLSA analysis, suggesting high conformational compatibility with the estrogen receptor. Tamoxifen showed more changes in MOLSA profiles, but luteoxanthin and 2,3-diphenylbenzofuran showed very consistent patterns. These findings demonstrate the phytochemicals' structural stability and potential as modulators of estrogen receptors (Figure 12).

**Figure 12: MOLSA Analysis Graph of the Selected Compounds and the Control Drug**



#### 4. Discussion

Luteoxanthin (−10.9 kcal/mol), 2,3-Diphenylbenzofuran (−10.2 kcal/mol),  $\alpha$ -Carotene (−10.3 kcal/mol), and sesaminol (−9.7 kcal/mol) were the top candidates with better binding affinities than tamoxifen, according to a computational screening of 1,346 IMPPAT phytochemicals against the estrogen receptor alpha (ER $\alpha$ , PDB ID: 3ERT). These substances established hydrophobic contacts and persistent hydrogen bonds with important residues (MET421, ASP451, LEU387, and ARG394) in the ER $\alpha$  ligand-binding pocket, indicating efficient regulation of receptor function. Their potential to inhibit estrogen-driven growth in endometrial cancer is demonstrated by their robust and enduring interactions, making them viable natural substitutes or supplements to traditional treatments. When compared to Tamoxifen, phytochemicals from traditional Indian medicinal plants showed better ER $\alpha$  binding, providing a natural and possibly safer substitute for synthetic modulators that have side effects and resistance. This strategy increases the range of endometrial cancer treatment options.

The top phytochemicals have positive ADME profiles, including high gastrointestinal absorption (>90%) and compliance with Lipinski's Rule of Five, indicating strong oral bioavailability, according to pharmacokinetic analyses using SwissADME and pkCSM. In comparison to traditional synthetic ER $\alpha$  modulators, toxicity projections showed low hepatotoxicity, cardiotoxicity, or mutagenicity, indicating a safer profile. These findings bolster their potential as well-tolerated and successful endometrial cancer treatment options.

The stability of the phytochemical-ER $\alpha$  complexes was shown using 100 ns molecular dynamics simulations. RMSF analyses (0.10–0.16 nm) revealed poor flexibility at important binding residues, although RMSD values (0.25–0.29 nm) suggested little structural drift. Robust ligand accommodation was demonstrated by a consistent radius of gyration (2.31–2.36 nm), steady SASA, PSA, and MOLSA values, and persistent hydrogen bonding, especially for 2,3-Diphenylbenzofuran. All of these results support the docking predictions and imply that these substances sustain steady receptor modification in physiological settings.

Experimental validation is necessary for these computational results. Future research should examine the phytochemicals' pharmacokinetics, safety, and efficacy both in vitro and in vivo, as well as any potential synergistic effects with current treatments.

#### 5. Conclusions

To treat hormone receptor-positive endometrial cancer, this study finds new natural modulators of estrogen receptor alpha (ER $\alpha$ ). Luteoxanthin, 2,3-Diphenylbenzofuran,  $\alpha$ -Carotene, and Sesaminol were shown to be the best candidates after computational screening of 1,346 phytochemicals from the IMPPAT database. These compounds had better binding affinities than Tamoxifen. The stability of these ligand-receptor complexes was verified by molecular dynamics simulations lasting more than 100 ns. RMSD, RMSF, and radius of gyration analyses showed preserved structural integrity. Their therapeutic potential was highlighted by a pharmacokinetic study utilizing SwissADME and pkCSM, which revealed high gastrointestinal absorption, favorable drug-likeness, and minimum toxicity. This strategy offers safer substitutes for synthetic ER $\alpha$  modulators by combining conventional medical knowledge with cutting-edge computational techniques, potentially resolving problems with medication resistance and side effects. The creation of successful, next-generation ER $\alpha$ -targeted treatments for endometrial cancer is made possible by these discoveries, which lay the groundwork for experimental

validation through in vitro and in vivo investigations as well as structure-activity relationship optimization.

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