

Molecular Docking-Based Screening of Epicatechin Gallate against Estrogen Receptor 1 (ESR1) Using Estradiol as a Standard Ligand: Insights into Female Reproductive Health

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Abstract

Female fertility and ovarian function are controlled by a complex network of hormonal signals, in which estrogen receptors play an important regulatory role. Among these receptors, estrogen receptor 1 (ESR1) is widely expressed in ovarian tissues and contributes to essential reproductive processes, including ovulation and maintenance of hormonal balance. In recent years, natural phytochemicals have attracted attention as potential regulators of estrogen receptor activity because they possess structures similar to natural estrogens and generally show good safety profiles.

In this study, potential phytochemical candidates were evaluated for their ability to interact with estrogen receptor 1 (ESR1; UniProt ID: P03372; PDB ID: 1A52), using Estradiol as a standard reference compound. Molecular docking analysis was carried out using LibDock and CDOCKER protocols available in BIOVIA Discovery Studio 2019. In addition, structural dynamics studies were performed to assess the stability and behavior of protein-ligand complexes.

The docking results showed that the selected phytochemicals demonstrated strong binding potential toward ESR1 by forming interactions with important amino acid residues within the receptor's ligand-binding region. When compared with the reference compound Estradiol, these compounds showed favorable binding patterns and stable conformations. Further structural dynamics analysis supported the stability of the formed complexes.

Overall, the findings indicate that the investigated phytochemical, Epicatechin Gallate (PubChem CID: 107905), may have the potential to influence ESR1-related signaling pathways involved in female reproductive health and ovarian physiology. This computational study provides valuable preliminary evidence for further experimental research toward the development of plant-based therapeutic approaches for reproductive health management.

Keywords: Estrogen Receptor 1 (ESR1), Female Fertility, Ovarian Physiology, Molecular Docking, Phytochemicals.

Introduction

Female reproductive health depends on the proper coordination of complex hormonal and molecular signaling pathways that regulate important processes such as ovarian development, follicle growth, ovulation, and embryo implantation. Among the various hormones involved in these processes, estrogen plays a central role in maintaining normal reproductive function. Estrogen carries out its biological effects by interacting with specific proteins known as estrogen receptors, which regulate the expression of several genes involved in reproductive physiology.

Two major types of estrogen receptors have been identified in mammals: estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2). Although both receptors share similar structural features and respond to estrogen stimulation, they differ in their distribution and biological functions in different tissues. ESR1 is highly expressed in reproductive organs, including the ovaries and uterus, as well as in tissues such as the mammary glands and brain. Due to its important role in estrogen-mediated signaling, ESR1 has received significant attention in studies related to female reproductive health.

Within the ovary, ESR1 regulates several essential processes, including follicular development, hormone production, and maintenance of reproductive balance. Proper ESR1 activity is necessary for normal ovarian function, while disturbances in ESR1 signaling may lead to changes in follicle development, altered steroid hormone production, and reduced fertility. Therefore, ESR1 has emerged as an important molecular target for identifying compounds that may help regulate estrogen-dependent reproductive pathways.

In recent years, natural plant-derived compounds, commonly known as phytochemicals, have gained increasing interest as potential alternatives for hormone-related therapeutic approaches. These compounds are structurally similar to naturally occurring estrogens and can interact with estrogen receptors. Many phytochemicals have demonstrated estrogen-like, antioxidant, and anti-inflammatory properties, making them valuable candidates for reproductive health research. Estradiol, a naturally occurring estrogen hormone, is widely used as a reference compound because of its well-characterized interaction with estrogen receptors and its established role in regulating reproductive functions.

The development of computational techniques has greatly improved the efficiency of early-stage drug discovery and biological research. Molecular docking is a widely used computational approach that helps predict how small molecules interact with target proteins by evaluating their binding orientation, interaction patterns, and binding strength. BIOVIA Discovery Studio 2019 provides advanced docking tools, including LibDock and CDOCKER, which allow researchers to analyze ligand-receptor interactions with high accuracy. Additionally, structural dynamics analysis provides further information about the stability and flexibility of protein-ligand complexes under simulated conditions.

The present study aimed to evaluate the interaction of selected phytochemicals with estrogen receptor 1 (ESR1; PDB ID: 1A52; Tanenbaum et al., 1998) using molecular docking and

structural dynamics approaches. Estradiol was used as a standard reference ligand to compare the binding efficiency and interaction characteristics of the 41 selected compounds. The findings of this computational investigation and screening may provide valuable insights into the potential role of natural compounds in regulating ESR1-mediated signaling pathways associated with female fertility and ovarian physiology, and may support future experimental studies for reproductive health applications.

Materials and Methods

Retrieval and Preparation of Protein Structure

The three-dimensional structure of human estrogen receptor 1 (ESR1; UniProt ID: P03372) was obtained from the Protein Data Bank (PDB) using the accession number 1A52 (Lussi et al., 2023; Bekker et al., 2025). The retrieved crystal structure was selected as the receptor model for computational analysis. The protein structure was imported into BIOVIA Discovery Studio 2019 (<https://discover.3ds.com/biovia-discovery-studio-simulation>) for further preparation and refinement before molecular docking studies.

Protein preparation is an essential step to ensure the accuracy and reliability of docking results. During this process, unnecessary water molecules and non-essential heteroatoms were removed from the protein structure to prevent unwanted interactions during ligand binding analysis. Missing hydrogen atoms were added to the receptor, and the CHARMM force field was applied to optimize the structural geometry. Subsequently, energy minimization was performed using the Smart Minimizer algorithm to remove structural inconsistencies and obtain a stable protein conformation suitable for docking simulations.

Ligand Selection and Preparation

The selected phytochemical compounds and the reference ligand Estradiol were retrieved from the publicly available chemical database PubChem (Marbán-González et al., 2025). The downloaded ligand structures were prepared using BIOVIA Discovery Studio 2019 to ensure their suitability for molecular docking analysis.

Ligand preparation involved generating three-dimensional conformations, correcting structural abnormalities, adjusting valence states, and applying appropriate protonation states under physiological conditions. The prepared ligands were further optimized using the CHARMM force field, followed by energy minimization to obtain stable, low-energy conformations. These optimized ligand structures were subsequently used for interaction studies with ESR1.

Identification of the Active Binding Site

The active binding region of ESR1 was determined based on the position of the co-crystallized ligand present in the protein structure. In addition, cavity detection tools available in BIOVIA Discovery Studio 2019 were used to identify potential ligand-binding regions within the receptor.

The selected binding pocket was defined as the docking site for all subsequent molecular docking analyses. The amino acid residues present within the ligand-binding domain were carefully analyzed to understand their possible contribution to ligand recognition and receptor interaction.

Molecular Docking Analysis Using LibDock

Initial docking studies were performed using the LibDock protocol available in BIOVIA Discovery Studio 2019. LibDock is a receptor-based docking method that identifies favorable ligand orientations by analyzing interactions between ligand conformations and receptor hotspot regions (Diller & Merz, 2001).

During docking simulations, multiple possible conformations of each ligand were generated and evaluated within the ESR1 binding pocket. The best docking pose was selected based on LibDock score, binding orientation, and the presence of favorable molecular interactions with important receptor residues. Higher docking scores and stronger interactions were considered indicators of better binding potential between the ligand and receptor. After high-throughput screening of candidate phytochemicals molecules based on the LibDock score of the reference molecule, molecular dynamics (MD)-based docking was subsequently performed for identification of most prominent candidate using CDOCKER module.

CDOCKER-Based Docking Validation

To further confirm the high-throughput docking results, the docked complexes were analyzed using the CDOCKER module of BIOVIA Discovery Studio 2019. CDOCKER is a CHARMm-based docking approach that uses molecular dynamics and simulated annealing techniques to generate optimized ligand conformations within the receptor binding site (Wu et al., 2003).

The stability and interaction strength of the docked complexes were evaluated using CDOCKER energy and CDOCKER interaction energy values. Compounds exhibiting lower interaction energy and favorable docking scores were considered to have stronger binding affinity and improved stability within the ESR1 active site.

Structural Dynamics and Stability Analysis

Structural dynamics analysis was performed to investigate the stability and conformational behavior of the ESR1-ligand complexes. The docked complexes were evaluated using important structural parameters, including root mean square fluctuation (RMSF), hydrogen bond interactions, and overall conformational stability.

These analyses provided insights into the flexibility of amino acid residues, persistence of ligand-receptor interactions, and the ability of the complexes to maintain stable binding throughout the simulation period. The obtained results were used to further assess the potential of selected phytochemicals as modulators of ESR1-mediated reproductive signaling pathways.

Results and Discussion

Preparation and Analysis of ESR1 Protein Structure

The three-dimensional structure of the ESR1 receptor obtained from the Protein Data Bank was successfully prepared and optimized for molecular docking analysis. Protein preparation and energy minimization steps resulted in the removal of structural irregularities, elimination of steric clashes, and improvement of receptor stability. The optimized ESR1 structure showed a well-defined ligand-binding domain with suitable structural characteristics for interaction with small molecules. The active binding region of ESR1 was analyzed to identify important amino acid residues involved in ligand recognition and receptor activation. The binding cavity demonstrated appropriate structural features to accommodate the reference ligand Estradiol as well as the selected phytochemical compounds. The active site architecture and identified residues confirmed that the receptor was suitable for further molecular docking investigations (Figure 1).

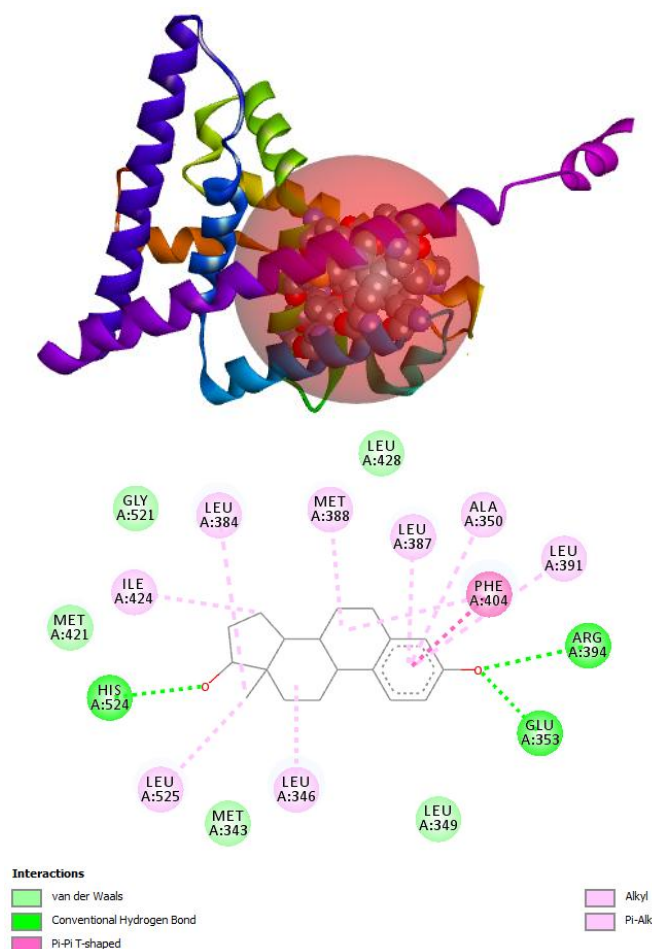


Figure 1: (a) Active site analysis of ESR1 receptor (b) Active site residues involved in ligand binding

Screening of Flavonoid Compounds against ESR1

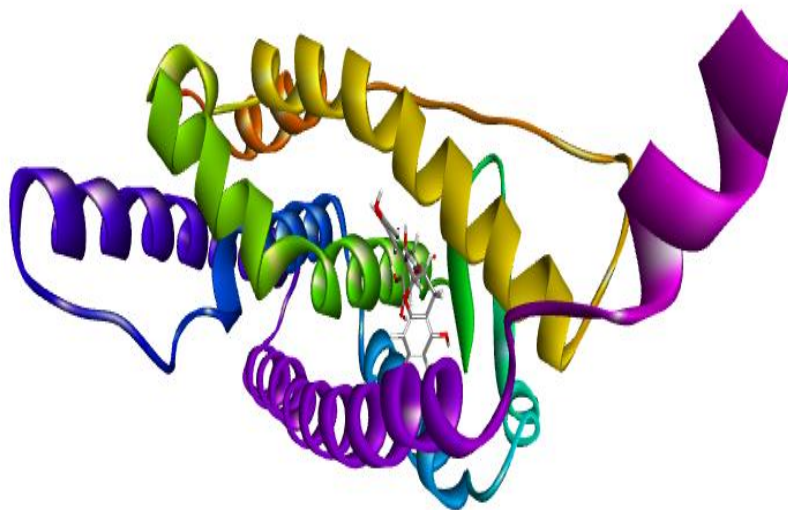
A total of 41 flavonoid compounds were selected and screened for their potential interaction with ESR1, while Estradiol was used as the reference ligand for comparative analysis (Table 1). These flavonoids were selected due to their reported biological activities, structural similarity to estrogenic compounds, and potential ability to interact with estrogen receptors.

Molecular docking analysis revealed that Epicatechin Gallate (PubChem CID: 107905) successfully occupied the ligand-binding cavity of ESR1 and demonstrated favorable interactions with important amino acid residues present within the receptor-binding domain. The interaction profile suggested that hydrogen bonding, hydrophobic interactions, and other non-covalent forces contributed significantly to the stability of the ESR1-Epicatechin Gallate complex (Figure 2).

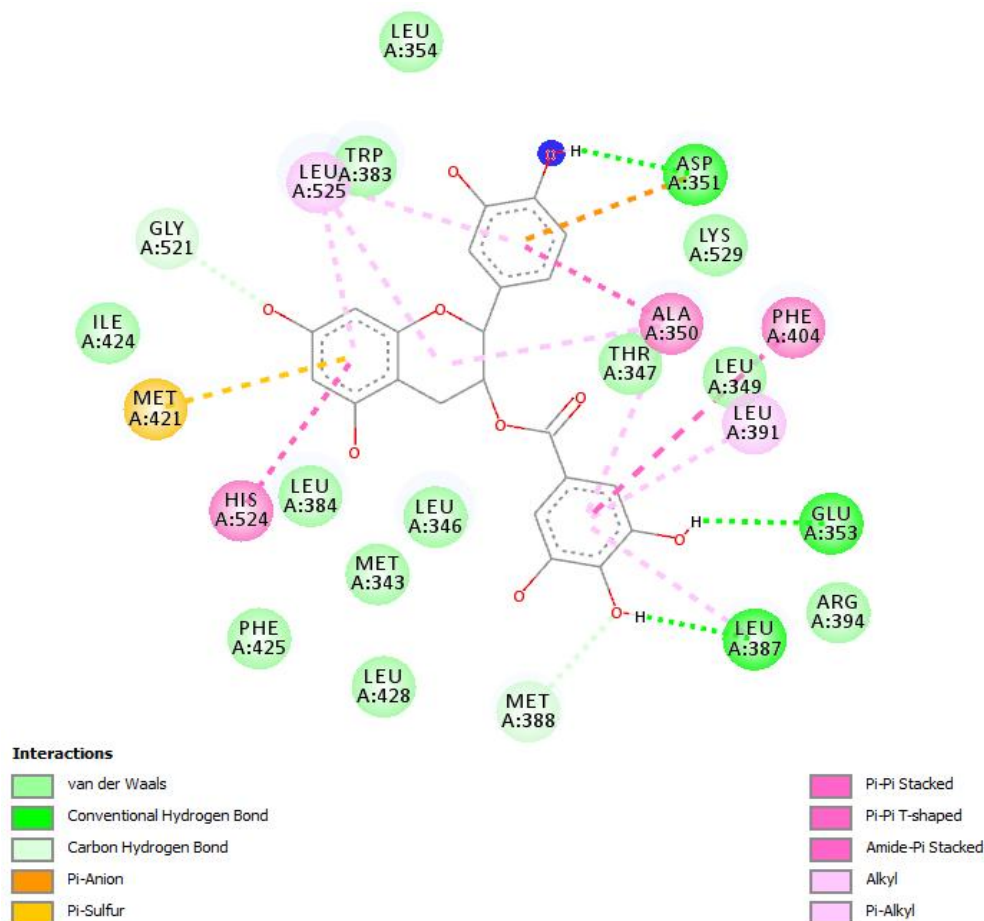
Table 1: List of 41 flavonoids screened against ESR1

S.N.	Flavonoids	PubChem CID
1.	Acacetin	5280442
2.	Apigenin	5280443
3.	Baicalein	5281605
4.	Biochanin A	5280373
5.	Catechin	9064
6.	Chrysin	5281607
7.	Cyanidin	128861
8.	Cyanidin-3-glucoside	441667
9.	Daidzein	5281708
10.	Delphinidin	128853
11.	Delphinidin-3-glucoside	443650
12.	Diosmetin	5281612
13.	Epicatechin	72276
14.	Epicatechin gallate	107905
15.	Epigallocatechin	72277
16.	Epigallocatechin gallate	65064
17.	Eriodictyol	440735
18.	Fisetin	5281614
19.	Formononetin	5280378
20.	Galangin	5281616
21.	Gallocatechin gallate	5276890
22.	Glycitein	5317750
23.	Hesperetin	72281
24.	Hesperidin	10621
25.	Isorhamnetin	5281654
26.	Kaempferol	5280863
27.	Luteolin	5280445
28.	Malvidin	159287
29.	Myricetin	5281672
30.	Naringenin	439246
31.	Naringin	442428
32.	Pelargonidin	440832
33.	Pelargonidin-3-glucoside	443648
34.	Peonidin	441773
35.	Petunidol anion	441774
36.	Procyanidin B1	11250133
37.	Procyanidin B2	122738
38.	Quercetin	5280343

39.	Scutellarin	185617
40.	Taxifolin	439533
41.	Wogonin	5281703



(a)



(b)

Figure 2: (a) Three-dimensional visualization of Epicatechin Gallate interaction with ESR1 receptor (b) Two-dimensional interaction profile

LibDock-Based Binding Analysis

The LibDock docking approach was employed to evaluate the binding compatibility of screened flavonoids within the ESR1 active site. Among the analyzed compounds, Epicatechin Gallate showed a higher LibDock score compared with the reference ligand Estradiol, indicating strong receptor compatibility and favorable ligand orientation.

The LibDock score obtained for Estradiol was 111.388, whereas Epicatechin Gallate demonstrated a higher score of 146.102 (Table 2). The improved docking score suggests that Epicatechin Gallate possesses strong geometric complementarity with the ESR1 binding pocket and may establish stable interactions with the receptor.

The higher docking score observed for Epicatechin Gallate may be attributed to the presence of multiple hydroxyl groups and aromatic structures that facilitate hydrogen bonding and hydrophobic interactions within the ligand-binding domain.

Table 2: LibDock docking scores of Estradiol and Epicatechin Gallate

S.N.	Molecule	LibDock Score
1	Control Estradiol (PubChem CID: 5757)	111.388
2	Epicatechin Gallate (PubChem CID: 107905)	146.102

CDOCKER Validation and Binding Energy Analysis

To further confirm the docking results obtained through LibDock analysis, CDOCKER-based molecular docking was performed. This method provided additional information regarding the energetic stability and interaction strength of the ligand-receptor complexes. The CDOCKER analysis showed that Epicatechin Gallate exhibited stronger interaction characteristics compared with Estradiol. The CDOCKER energy value for Epicatechin Gallate was 17.2529, while Estradiol showed a value of 9.74342. Similarly, the binding energy of Epicatechin Gallate (-133.9435 kcal/mol) was more favorable than that of Estradiol (-90.7729 kcal/mol) (Table 3 & Table 4). These results indicate that Epicatechin Gallate formed a stable interaction with ESR1 and may have a strong binding potential within the receptor cavity. The comparative docking results suggest that this phytochemical could act as a promising natural modulator of ESR1-mediated signaling pathways.

Table 3: CDOCKER energy and binding energy analysis

S.N.	Molecule	-CDOCKER Energy	Binding Energy (kcal/mol)
1	Control Estradiol (PubChem CID: 5757)	9.74342	-90.7729
2	Epicatechin Gallate (PubChem CID: 107905)	17.2529	-133.9435

Molecular Interaction Analysis

Detailed interaction analysis revealed that Epicatechin Gallate formed several important interactions with residues located within the ESR1 ligand-binding domain. These interactions included hydrogen bonding, van der Waals forces, π - π stacking interactions, and hydrophobic contacts, which collectively contributed to the stabilization of the receptor-ligand complex.

The interaction pattern of Epicatechin Gallate showed similarities with the Estradiol-ESR1 complex, indicating that the compound may recognize the receptor binding region through comparable molecular mechanisms. The ability of Epicatechin Gallate to interact with key residues involved in ligand recognition suggests its potential role in influencing ESR1 activity.

Table 4: Binding energy and interaction analysis details

Ligand Name	Binding Energy (kcal/mol)	Ligand Energy (kcal/mol)	Protein Energy (kcal/mol)	Complex Energy (kcal/mol)	Entropic Energy (kcal/mol)
107905	-133.9435	-28.6979	-2051.3154	-2213.9567	20.4310
EST	-90.7729	23.3913	-2051.3154	-2118.6970	19.0551

Structural Dynamics and Stability Analysis

Structural dynamics analysis was conducted to evaluate the stability and conformational behavior of the ESR1-Epicatechin Gallate complex. The analysis included evaluation of solvation behavior, root mean square fluctuation (RMSF), and energy stability parameters. The RMSF analysis demonstrated minimal fluctuations in the active-site residues of the receptor, indicating that binding of Epicatechin Gallate did not cause major structural disturbances. The stable RMSF profile suggested that the ligand remained well accommodated within the ESR1 binding pocket throughout the simulation period. Furthermore, consistent energy profiles and stable conformational patterns supported the overall stability of the docked complex. These findings indicate that Epicatechin Gallate maintains stable interactions with ESR1 and may possess the ability to regulate receptor-mediated biological pathways (Figure 3; Table 5).

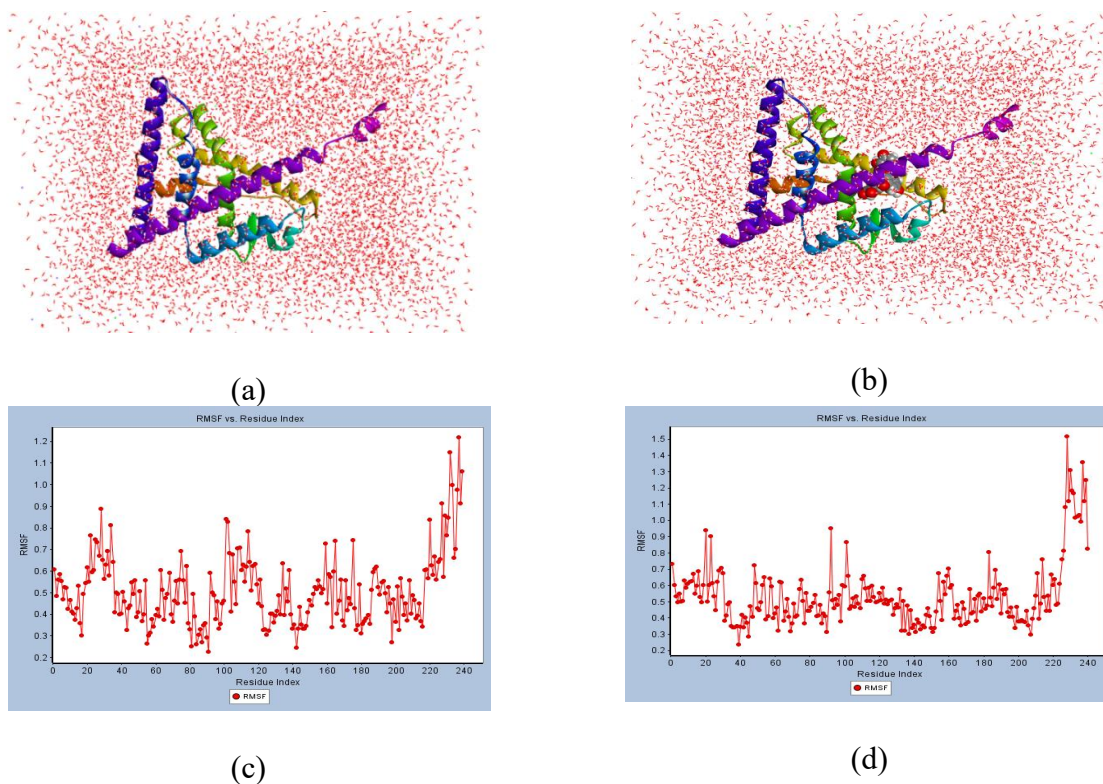


Figure 3: (a) ESR1 solvation analysis (b) Epicatechin Gallate-ESR1 complex solvation analysis (c) RMSF analysis of ESR1 (d) RMSF analysis of Epicatechin Gallate-ESR1 complex

Table 5: Standard dynamics cascade energy analysis of ESR1 and Epicatechin Gallate-ESR1 complex

Name	Stage	Forcefield	Start Time (ps)	End Time (ps)	Initial Potential Energy (kcal/mol)	Total Energy (kcal/mol)	Potential Energy (kcal/mol)	Kinetic Energy (kcal/mol)	Temperature (K)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Å))	Final RMS Gradient (kcal/(mol x Å))
1A52	Minimization	CHARMm			53497221.999		-85810.349			3154.701	-75252.565	628781.936	0.989
1A52	Minimization2	CHARMm			-85810.349		-101108.628			8397.231	-92746.484	0.989	0.060
1A52	Heating	CHARMm	0.000	4.000	-101108.628	-68290.882	-83047.096	14756.214	285.696	4890.429	-73658.546	16.696	16.763
1A52	Equilibration	CHARMm	4.000	14.000	-83047.096	-67776.518	-83446.159	15669.640	303.381	4496.117	-73143.992	16.763	16.906
1A52	Production	CHARMm	14.000	24.000	-83446.159	-68330.545	-83930.793	15600.247	302.037	4332.110	-73215.312	16.906	16.839

(a)

Name	Stage	Forcefield	Start Time (ps)	End Time (ps)	Initial Potential Energy (kcal/mol)	Total Energy (kcal/mol)	Potential Energy (kcal/mol)	Kinetic Energy (kcal/mol)	Temperature (K)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Initial RMS Gradient (kcal/(mol x Å))	Final RMS Gradient (kcal/(mol x Å))
1A52+107905	Minimization	CHARMm			369401138.510		-86616.248			3284.403	-76072.115	33312468.510	0.976
1A52+107905	Minimization2	CHARMm			-86616.248		-101439.874			8567.433	-93190.862	0.976	0.090
1A52+107905	Heating	CHARMm	0.000	4.000	-101439.874	-68243.573	-83080.201	14836.628	285.834	4892.262	-73717.384	16.703	16.781
1A52+107905	Equilibration	CHARMm	4.000	14.000	-83080.201	-67654.344	-83433.356	15779.012	303.990	4156.587	-72918.635	16.781	16.818
1A52+107905	Production	CHARMm	14.000	24.000	-83433.356	-69041.419	-84645.240	15603.821	300.614	4199.738	-73806.482	16.818	16.789

(b)

Biological Significance of Epicatechin Gallate Interaction with ESR1

The overall computational findings suggest that Epicatechin Gallate exhibits strong binding affinity toward ESR1 and may influence estrogen-dependent signaling pathways associated with female reproductive physiology. Epicatechin Gallate (ECG), a naturally occurring catechin derivative, is known for its antioxidant and protective biological properties (Kamal et al., 2021; Rahman et al., 2018). The antioxidant activity of ECG may contribute to maintaining reproductive cellular health by reducing oxidative stress-related damage. Previous experimental studies have suggested that ECG may provide beneficial effects in reproductive and cellular systems; however, most available evidence is derived from *in vitro* and animal studies. Therefore, further experimental validation and clinical investigations are required to confirm its biological role in female reproductive health. Overall, this study provides computational evidence supporting Epicatechin Gallate as a potential natural ESR1 modulating compound. The findings may serve as a foundation for future experimental studies aimed at exploring plant-derived molecules for reproductive health applications.

Conclusion

The present study provides a computational evaluation of the interaction potential of the selected phytochemical Epicatechin Gallate with estrogen receptor 1 (ESR1; PDB ID: 1A52), using Estradiol as a reference ligand. Molecular docking analyses performed using LibDock and CDOCKER approaches revealed that Epicatechin Gallate showed strong binding potential and

established favorable interactions within the ESR1 ligand-binding domain. The observed hydrogen bonding, hydrophobic interactions, and other molecular contacts contributed to the stability of the receptor-ligand complex.

Structural dynamics analysis further supported the docking results by demonstrating that the Epicatechin Gallate-ESR1 complex maintained structural stability and showed minimal conformational fluctuations during the simulation period. Comparative evaluation with Estradiol suggested that Epicatechin Gallate may have the ability to interact effectively with ESR1 and could serve as a potential natural modulator of estrogen-mediated signaling pathways.

Overall, the findings highlight the possible role of Epicatechin Gallate as a promising plant-derived bioactive compound associated with female reproductive health and ovarian physiological regulation. This computational investigation provides preliminary evidence that may support the future development of natural therapeutic strategies targeting estrogen receptor-related pathways. However, further experimental validation through in vitro studies, animal models, and clinical investigations is necessary to confirm its biological activity, safety, and therapeutic potential.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

Acknowledgments

The authors acknowledge BIOVIA Discovery Studio 2019 for the computational platform used in this study. The authors also thank publicly accessible structural and chemical databases (PDB & PubChem) for making biological data available for research purposes.

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