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INHIBITION OF ESTROGEN RECEPTOR ALPHA (ERα) BY BIOACTIVE COMPOUNDS FROM *TERMINALIA ARJUNA* (Roxb. ex DC.) Wight & Arn.: A MOLECULAR DOCKING STUDY

Padmavathy Balachandran[1], *Sathish Muthukrishnan*[2*], *Samuel Ebinezer Balakrishnan*[1]

Address(es):
[1] Department of Physics, Government Arts College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), Kumbakonam-612002.
[2*] Department of Microbiology, JJ College of Arts and Science (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli), Pudukkottai– 622 422, Tamil Nadu, India.

*Corresponding author: sathishkrish55@gmail.com

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ABSTRACT

Estrogen receptor alpha (ERα) plays a critical role in breast cancer. Its overabundance can be driven by factors that stimulate estrogen hormone gene expression in living organisms. This, in turn, can lead to the development of several desirable properties by the cancer cells, impairing the maintenance of a regular mammary gland in females. Consequently, ERα offers a wide range of potential biochemical therapeutic targets for clinical research. *Terminalia arjuna*, a widely accepted medicinal plant in traditional medicine, has shown promise in treating various critical diseases. Our previous studies using swissADME identified 20 bioactive compounds in *T. arjuna* with favorable pharmacokinetic properties. This study aimed to evaluate the potential of these bioactive compounds against ERα (PDB ID: 3ERT) using molecular docking studies with Autodock 4.2.6. The docking results revealed high binding affinities for the designed compounds, ranging from -3.1 to -9.4 kcal/mol. These findings suggest that *T. arjuna* derived compounds could be significant for the development of novel and improved anti-breast cancer agents.

Keywords: Autodock, Binding Affinity, Breast cancer protein, Estrogen Receptor Alpha, Molecular Docking, *Terminalia arjuna*

INTRODUCTION

Traditional medicinal plants have gained renewed interest in recent years due to their significant role in treating various human illnesses around the globe **(Bhardwaj *et al.*, 2021)**. The World Health Organization (WHO) estimates that over 80% of people in developing countries rely primarily on traditional plant-based medicines for their basic healthcare needs (**Amrati *et al.*, 2021)**. This continued interest stems from the vast array of medicinal properties found in plants, including antimicrobial, antioxidant, and anti-inflammatory effects. Additionally, plants offer benefits like managing diabetes, nausea, malaria, fertility, asthma, stress, and even cancer **(Tran *et al.*, 2020)**. The therapeutic power of these plants lies in their unique bioactive compounds. Plants produce a wide variety of chemicals, many with the potential to become new pharmaceuticals. These compounds have a definite physiological effect on the human body **(Larayetan *et al.*, 2019)**. For instance, Ayurvedic medicine highly regards *Terminalia arjuna* as a vital herb for treating various ailments (**Petrovska, 2012)**. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. (Arjun tree), a deciduous giant native to India, reaches heights of 20-30 meters and belongs to the Combretaceae family. Well-regarded in Ayurveda, ancient Indian medicine, for its use in heart disease. Ayurvedic texts describe the tree's bark as having various properties (**Kumar *et al.*, 2016)**. *T. arjuna* has been employed as a cardiotonic in the management of heart failure, ischemic cardiomyopathy, atherosclerosis, and myocardial necrosis. It has also been utilized in treating various human diseases, including blood disorders, anemia, venereal and viral infections. Additionally, it has been used to promote overall health (**Kumar et al., 2013**). Beyond this, *T. arjuna* has been applied in the treatment of fractures, ulcers, and hepatic conditions. The plant has demonstrated hypocholesterolemic, antibacterial, antimicrobial, antitumoral, antioxidant, antiallergic, antifeedant, antifertility, and anti-HIV properties (**Bachaya *et al.*, 2009**). *T. arjuna* is reported to possess potent hydrolipidemic properties. It is believed that the saponin glycosides within *T. arjuna* might be responsible for its inotropic effects, while the flavonoids/phenolics could contribute to antioxidant and vascular amplification activities, thus supporting the plant's multifaceted cardioprotective role (**Amalraj and Sreeraj, 2017**).

Cancer is a group of diseases defined by abnormal cells growing uncontrollably and spreading throughout the body. Breast cancer is a particularly common and aggressive form of cancer that disrupts the normal function of breast cells. Among women globally, breast cancer stands as both the most common cancer besides skin cancer and a major cause of cancer death. According to studies, one in ten women globally will develop breast cancer at some point in their lives **(Yedjou *et al.*, 2019; Torre *et al.*, 2015)**. Excessive estrogen production is considered a major risk factor for Breast cancer. Estrogen receptors (ERs) are proteins found in cells that are activated by the hormone estrogen. There are two main types, ER-alpha and ER-beta, which play a role in regulating cell growth and development. ERα is particularly abundant in breast tissue and the uterus **(Paterni et al., 2014; Rafeeq, 2022)**.

In the quest for new drugs with minimal side effects, molecular docking has become a cornerstone of computational drug discovery. This technique acts like a virtual screening tool, predicting how a test molecule fits into the binding site of a targeted receptor protein. Ideally, a docking method should accurately assess the binding strength between the molecule (ligand) and the receptor complex. Molecular docking studies have proven valuable in pinpointing the specific interaction sites between bioactive compounds and their protein targets, aiding in the discovery and development of new medications (**Abdulfatai *et al.*, 2017; Paul *et al.*, 2018; Thamaraiselvi *et al.*, 2021**).

In our previous study, ADMET (Adsorption, Distribution, Metabolism and Toxicity) Prediction of 20 Bioactive Compounds in *T. arjuna*, were exhibited promising properties for pharmacokinetics, bioavailability, drug likeness, and gastrointestinal absorption **(Padmavathy and Samuel, 2023)**. The present study was aimed to evaluate the anticancer potential of bioactive compounds in *T. arjuna* against ERα Protein conducted by molecular docking studies.

MATERIALS AND METHODS

Protein Preparation

We retrieved the 3D crystal structure of the estrogen receptor alpha (ER-α) (PDB ID: 3ERT) (Figure 1) from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/structure/3ert). Since the original structure of ERα represents a homo2-mer, we isolated a single protein chain to create a monomer for further analysis using docking studies. The protein was prepared for docking using the Biovia Discovery Studio **(Sathish *et al.*, 2024).**

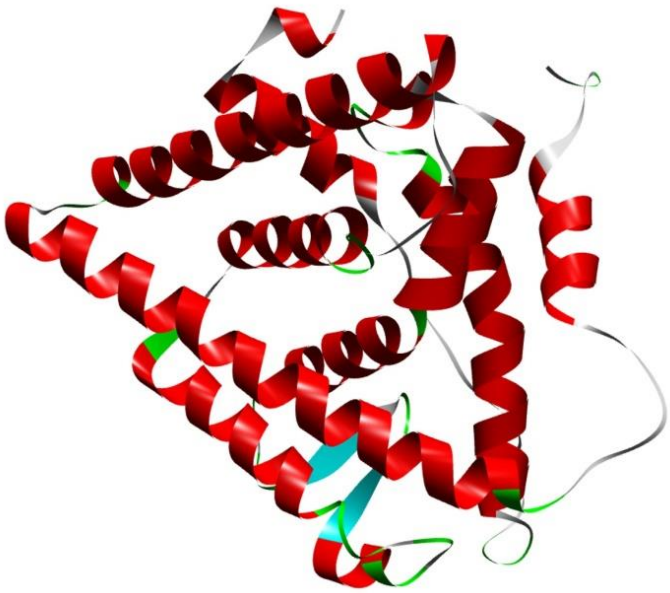

Figure 1 3D Structure of Estrogen Receptor Alpha (ERα)

Ligand Preparation

The 3D structures of bioactive compounds (Figure 2) were retrieval our previous study revealed twenty bioactive compounds (**Padmavathy and Samuel, 2023)**. Namely (1) Anethole; (2) 1,1-Diethoxy-3-methylbutane; (3) S)-(+)-2-Amino-3-methyl-1-butanol; (4) Ethyl pipecolinate; (5) Glycerin; (6) Pentanoic acid; (7) Cyclopropanecarboxylic acid, 1-amino-; (8) Benzeneacetaldehyde; (9) Glycine, N-(2-methyl-1-oxo-2-butenyl)-, methyl ester, (E)-; (10) 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; (11) 1-Methoxyhexane; (12) 9-Oximino 2,7-diethoxyfluorene; (13) Heptadecane, 9-hexyl; (14) 9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol; (15) Butanoic acid, 2,3 dihydroxypropyl ester; (16) 2-Naphthalene methanol; (17) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) ; (18) 3-Hydroxyspirost-8-en-11-one; (19) Protriptyline; and (20) Olean-12-ene-3,15,16,21,22,28-hexol, (3α,15α,16α,21α,22α) from PubChem's SDF files were converted to PDB format via Open Babel **(Surendirakumar *et al.*, 2023)**.

Molecular docking Studies

To understand the interactions between a target protein and potential drug molecules (ligands), we employed molecular docking analysis to determine the most favorable binding geometry of the protein-ligand complex. This technique is crucial in drug discovery. In this study, the ERα served as the target protein (Fig 1). The crystal structure of ERα was obtained from the PDB. Water molecules and any previously bound ligands were removed. Using AutoDock 4.2.6. software, Kollman charges were assigned to the protein, missing atoms were repaired, and hydrogen atoms were added. The prepared protein structure was saved in PDBQT format. Similar to the protein, Kollman and Gasteiger charges were calculated for the ligand molecules. Torsion bonds were identified, and some were set as rotatable or non-rotatable depending on their flexibility. The prepared ligand structures were saved in PDBQT format, containing information about partial charges and atom types. AutoDock's grid module was used to define the search space around the active site of the protein where the ligand would dock. This involved specifying the grid dimensions and center. AutoDock 4.2.6. was employed to perform the docking studies. A configuration file was used to specify the protein, ligand, and grid parameters. The software generated log files and PDBQT files containing energy models for each ligand pose. The output files were analyzed to identify the lowest energy conformation for each ligand, representing the most stable binding pose. These poses were then visualized and analyzed using Biovia Discovery Studio to understand the specific interactions between the ligand and the protein's active site (**Sathish *et al.*, 2024; Surendirakumar *et al.*, 2023).**

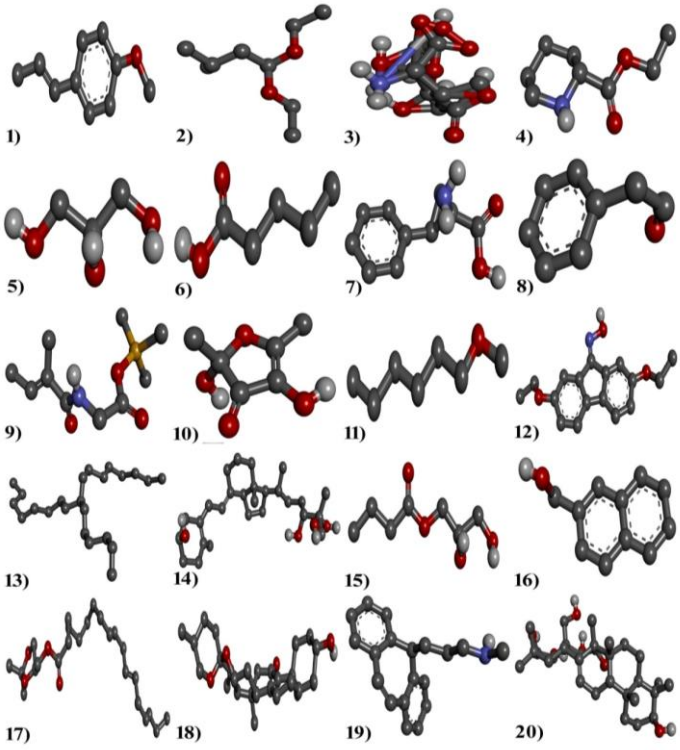

Figure 2 3D structure of Bioactive compounds from *T. arjuna* (1) Anethole; (2) 1,1-Diethoxy-3-methylbutane; (3) S)-(+)-2-Amino-3-methyl-1-butanol; (4) Ethyl pipecolinate; (5) Glycerin; (6) Pentanoic acid; (7) Cyclopropanecarboxylic acid, 1-amino-; (8) Benzeneacetaldehyde; (9) Glycine, N-(2-methyl-1-oxo-2-butenyl)-, methyl ester, (E)-; (10) 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; (11) 1-Methoxyhexane; (12) 9-Oximino 2,7-diethoxyfluorene; (13) Heptadecane, 9-hexyl; (14) 9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol; (15) Butanoic acid, 2,3 dihydroxypropyl ester; (16) 2-Naphthalene methanol; (17) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) ; (18) 3-Hydroxyspirost-8-en-11-one; (19) Protriptyline; and (20) Olean-12-ene-3,15,16,21,22,28-hexol, (3α,15α,16α,21α,22α)

RESULT AND DISCUSSION

Bioactive compounds from *T. arjuna* were computationally docked into the active site of ERα using AutoDock 4.2.6. Table 1 summarizes their binding affinities to the target protein. These affinities varied considerably, ranging from -3.1 to -9.4 kcal/mol. As shown in Figure 3, five compounds displayed particularly strong binding. 3-Hydroxyspirost-8-en-11-one emerged as the top candidate with the highest binding affinity (-9.4 kcal/mol) achieved through a hydrogen bond interaction with LEU536. Olean-12-ene-3,15,16,21,22,28-hexol (3α,15α,16α,21α,22α) followed closely with a binding affinity of -8.64 kcal/mol, forming two hydrogen bonds, one with ASP351 and another with CYS530. Protriptyline displayed a binding affinity of -7.8 kcal/mol and interacted with a single amino acid, VAL534, via a hydrogen bond. Interestingly, 9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol, despite exhibiting a binding affinity of -7.5 kcal/mol, lacked any hydrogen bond interactions with the target protein. Finally, 2-Naphthalene methanol displayed a binding affinity of -6.5 kcal/mol and formed two hydrogen bond interactions, one with LEU346 and another with GLU353.

Molecular docking has become a best in the field of inhibitor discovery. It allows researchers to virtually explore and predict how inhibitor molecules interact with their target proteins **(Ahirwar *et al.*, 2016)**. This technique provides valuable insights into potential inhibition mechanisms and the nature of these interactions. In docking studies, the binding energy between the ligand (inhibitor molecule) and the protein complex is a key indicator of interaction strength **(Rafeeq, 2022)**. Our study identified promising inhibitor candidates with good binding affinity. These inhibitors interacted favorably with specific amino acids in the target protein's active site, including LEU536, ASP351, CYS530, VAL534, LEU346, and GLU353. Hydrogen bonds (H-bonds) formed between the inhibitors and these amino acids likely play a crucial role. H-bonds contribute significantly to the stability of the "inhibitor-protein" complex, ultimately influencing the inhibitor's potency (**Rafeeq, 2022).**

Table 1 ERα interaction with Bioactive Compounds from *T. arjuna*

S.No	NAME OF COMPOUNDS	Binding Affinity (kcal/mol)	No. Of Hydrogen Bond	Name of Hydrogen Bond	Distance Å
1.	Anethole	-5.1	0	-	-
2.	1,1-Diethoxy-3-methylbutane	-3.4	1	GLN375	3.20
3.	S)-(+)-2-Amino-3-methyl-1-butanol	-3.2	0	-	-
4.	Ethyl pipecolinate	-4.0	0	-	-
5.	Glycerin	-3.5	3	GLU385 ASN455 ARG515	2.70 4.01 3.00
6.	Pentanoic acid	-4.3	1	LEU391	3.94
7.	Cyclopropanecarboxylic acid, 1-amino-	-5.3	0	-	-
8.	Benzeneacetaldehyde	-4.7	1	ARG434	3.17
9.	Glycine, N-(2-methyl-1-oxo-2-butenyl)-, methyl ester, (E)-	-3.1	0	-	-
10.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	-4.9	2	GLU353 ARG394	2.90 4.02
11.	1-Methoxyhexane	-3.8	0	-	-
12.	9-Oximino 2,7-diethoxyfluorene	-6.3	1	LEU536	3.20
13.	Heptadecane, 9-hexyl	-4.6	0	-	-
14.	9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol	-7.5	0	-	-
15.	Butanoic acid, 2,3 dihydroxypropyl ester	-5.0	1	LYS449	2.96
16.	2-Naphthalene methanol	-6.5	2	LEU346 GLU353	2.12 2.54
17.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-4.3	0	-	-
18.	3-Hydroxyspirost-8-en-11-one	-9.4	1	LEU536	3.08
19.	Protriptyline	-7.8	1	VAL534	3.10
20.	Olean-12-ene-3,15,16,21,22,28-hexol,(3α,15α,16α,21α,22α)	-8.6	2	ASP351 CYS530	3.83 3.67

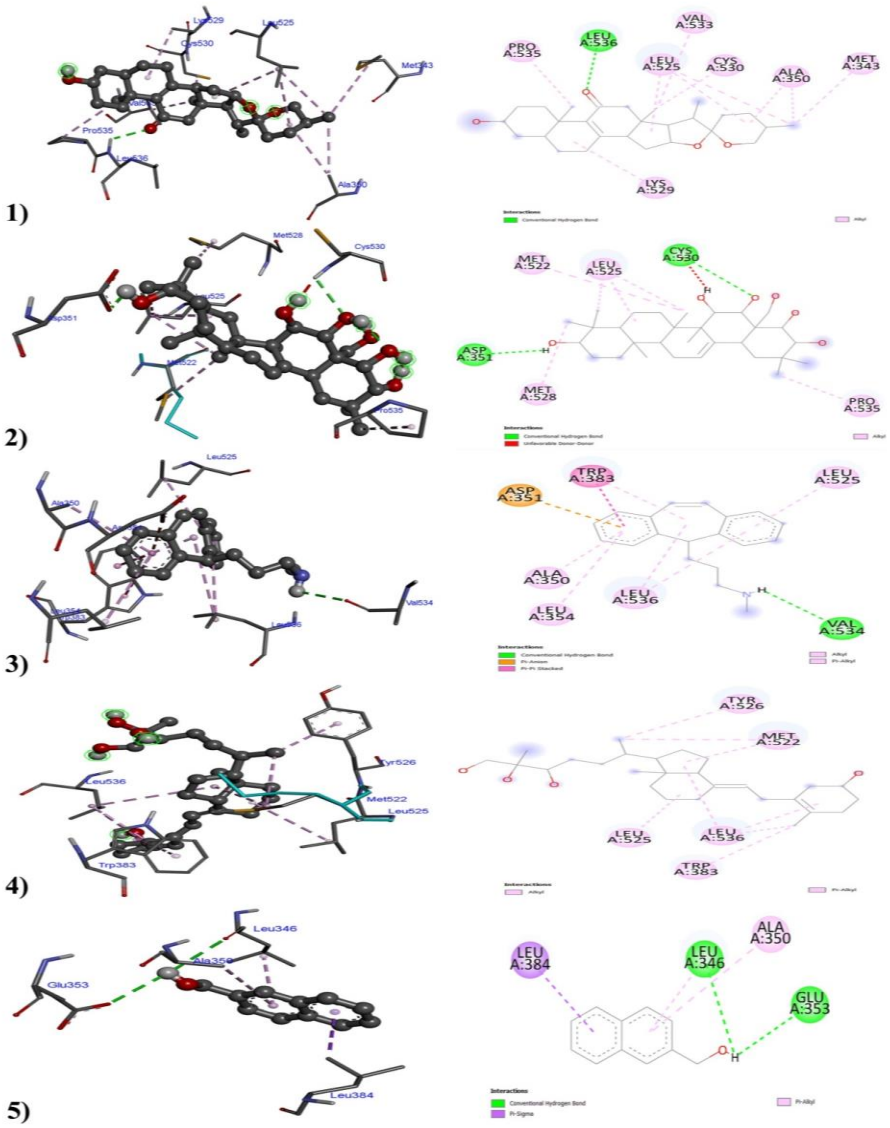

Figure 3 3D and 2D Structure of ERα against Bioactive Compounds (1) 3-Hydroxyspirost-8-en-11-one (2) Olean-12-ene-3,15,16,21,22,28-hexol (3α,15α,16α,21α,22α); (3) Protriptyline; (4) 9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol; (5) 2-Naphthalene

CONCLUSION

In the fight against breast cancer, one of the most common cancers among women globally, researchers are utilizing computational techniques to discover new drugs. This study employed molecular docking to analyze the interaction between protein molecule ERα and bioactive compounds from *T. arjuna*, a potential approach for breast cancer therapy. The results demonstrated significant binding between the tested compounds and ERα. Notably, compounds like 3-Hydroxyspirost-8-en-11-one, Olean-12-ene-3,15,16,21,22,28-hexol (3α,15α,16α,21α,22α), Protriptyline, 9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol, and 2-Naphthalene methanol exhibited the strongest interactions with ERα. Therefore, the findings from this investigation hold promise for the development of novel and improved therapeutic agents for breast cancer.

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