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Exploring the Role of *Ginkgo biloba* in Investigating 7L1X in Triple-Negative Breast Cancer Through Integrative Bioinformatics

Vignesh Gopinath*

Abstract

Objectives: Triple-negative breast cancer is a very dangerous form of cancer that affects mostly Hispanic and African American women above the age of 40. It represents approximately 15–20% of the global cancer incidence. This experiment aims to investigate the use of Gingko biloba in the treatment of the disease triple-negative breast cancer. Methods: We use a variety of online tools, and websites, including the Indian Medicinal Plants, Phytochemistry, and Therapeutics database, to extract the phytochemical compounds, the PubChem website to obtain the canonical SMILES, and the 2-D format is used to download the SDF files, the protein database website to download the protein files in protein database format, the Ramachandran's Plot. This way, we can visualize that in Discovery Studio BIOVIA app. Then, the Swiss ADME website is used to find which compounds satisfy Lipinski's rule, and the other conditions. Result: The results showed that the compounds Afzelin, D-Glucuronic acid, Gingketin, Acacetin, Quercetin, Isoginkgetin, and Genkwanin satisfy all the criteria, and could be further used in the treatment of TNBC. Conclusion: The observations suggest that the below-mentioned phytochemical compounds: Afzelin, D-Glucuronic acid, Gingketin, Acacetin, Quercetin, Isoginkgetin, and Genkwanin seem to satisfy all the conditions for it to be used in the ADME testing and analysis and can be used in the treatment of TNBC.

Keywords: Triple-negative breast cancer (TNBC), breast cancer gene-1 (BRCA), CK2 alpha kinase, Ginkgo biloba, Ramachandran's Plot

INTRODUCTION

TNBC is a heterogeneous type of cancer characterized by the absence of three critical receptors: ER, PR, and HEGFR-2 [1]. It is observed mainly in young African American women and Hispanic women carrying a mutation in the BRCA gene. It has a different molecular profile, is quite aggressive, and does not have many therapies [2]. It accounts for about 1,70,000 cases of the total worldwide cancer burden, which is about 15–20% of the total cancer burden [3, 4].

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Breast cancers are categorized into seven main subtypes: luminal A (characterized by ER positivity and low histologic grade), luminal B (ER-positive with high histologic grade), HER2 overexpression, basal-like (which includes two types, BL1 and BL2), immunomodulatory (IM), mesenchymal (M), and mesenchymal stem-like (MSL). Additionally, there is the usual breast-like subtype [3]. Many TNBCs fall into the basal-like subtype, though they display variability in gene expression profiles and immunohistochemistry

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(IHC) results. Basal-like breast cancer is defined by its gene expression profile, characterized by low expression of ER, PR, and HER2, alongside elevated expression of markers, such as CK5, CK14, caveolin-1, CAIX, p63, and EGFR (HER1). This expression pattern is attributed to the basal or myoepithelial cell component of the mammary gland [5].

TNBC and other aggressive forms of breast cancer demand targeted therapeutic approaches due to their unique molecular characteristics and resistance to conventional hormone therapies. These cancer cells can be killed by using small interfering RNAs (siRNAs) to block the production of survival genes. A survival protein kinase named cyclin-dependent kinase 11 (CDK11) controls RNA transcription, splicing, and mitosis. A survival protein kinase called casein kinase 2 (CK2) inhibits the demise of cancer cells. Reducing the production of these genes may be useful in treating breast cancer [6].

TNBC is widely known for its aggressive behavior and is distinguished by high mean tumor size, higher grade tumors, early onset, and occasionally a greater node positivity rate [7]. This group is also known for developing more aggressive metastases, which are more prone to occur in visceral organs, particularly the brain, and lungs, while being less likely to spread to bone [8]. These cancers are also characterized by an early peak in recurrence, typically occurring between the first and third year after diagnosis. The majority of TNBCs are ductal in origin, according to histological studies; however, apocrine, adenoid cystic, and metaplastic tumors are among the other aggressive phenotypes that also seem to be overrepresented [9]. An analysis of the histology of basal-like tumors, all of which were ER/HER2 negative, revealed a significant increase in mitotic count, geographic necrosis, pushing invasion borders, and a stromal lymphocytic response [10].

Conventional treatment options for breast cancer include radiation therapy, chemotherapy, and surgery. However, some individuals may also seek natural treatments to help manage symptoms or reduce their risk of developing breast cancer in the first place [11].

A popular remedy in recent times is *Ginkgo biloba*, an herbal supplement derived from the leaves of the ginkgo tree. Although *G. biloba* is mainly recognized for its possible advantages in the areas of cognition and circulation, certain research has also indicated that it might possess anti-cancer characteristics, particularly with breast cancer [12].

LITERATURE REVIEW

TNBC is a subtype of breast cancer defined by the lack of ERs, PRs, and HER2 expression. This absence makes TNBC difficult to treat with conventional hormones or targeted therapies. Researchers have focused on alternative treatments, and bioactive compounds from natural sources, such as *G. biloba*, have garnered interest due to their potential therapeutic properties.

G. biloba is a plant known for its neuroprotective, anti-inflammatory, and antioxidant properties, attributed to its rich composition of flavonoids, terpenoids, and phenolic acids. Some studies suggest that these bioactive compounds may exhibit anticancer activity by modulating pathways involved in cell proliferation and apoptosis. For TNBC, these properties could offer novel therapeutic avenues where conventional treatments fall short.

7L1X, a molecular target of interest, has been investigated in the context of TNBC to understand its role in cell signaling and cancer progression. Recent studies employing integrative bioinformatics approaches have shown that 7L1X may interact with several molecular networks associated with cancer cell proliferation, survival, and metastasis. By combining bioinformatics tools, such as gene expression profiling and protein interaction mapping, researchers can better understand the mechanistic action of *G. biloba* compounds on 7L1X and their broader effects on TNBC pathways.

Integrative bioinformatics allows for the in-depth analysis of complex datasets, providing insights into molecular interactions and potential biomarkers for TNBC. By analyzing these data, researchers

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are identifying promising leads for therapeutic targets and understanding how *G. biloba* compounds may influence these targets. This multidisciplinary approach offers potential pathways to novel therapeutic interventions for TNBC, highlighting *G. biloba*'s role as a candidate for further investigation in cancer therapy [13, 14].

MATERIALS AND METHODS

Selection and Preparation of the Target Protein

The 3D structure of the target receptors (7L1X) protein was downloaded in the PDB format from the RCSB Protein Data Bank (https://www.rcsb.org/). We visualize the protein using the Discovery Studio 2021 v21.1.0.20298 BIOVIA tool (https://discover.3ds.com) and water molecules, ions, and ligands removed.

Secondary Structure Prediction

"Secondary structure prediction" encompasses a set of bioinformatics methods aimed at forecasting the secondary structures of proteins and nucleic acids using only information from their primary sequences. Predicting the creation of protein structures like alpha helices and beta strands is what this means for proteins. This is done using the PDBSum website (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) [15].

The Ramachandran Plot is an approach to displaying energetically permitted regions of backbone dihedral angles ψ against φ of amino acid residues in the structure of proteins.

Ligand Selections

The phytochemical compounds are selected from the IMPPAT database (https://cb.imsc.res.in/imppat/). From there, canonical SMILES are obtained. Then, we paste the individual compounds into the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), and the SDF files are downloaded in the 2D format.

ADME Analysis

Then, the list of 20 Canonical SMILES is pasted into the Swiss ADME (http://www.swissadme.ch/index.php) website. From there, one can find out whether they satisfy the Lipinski rule. The conditions to be followed are that the GI absorption should be high, BBB permanent should be low, the GI should be low, and the Lipinsiki's rule should be 0 with no violations.

Molecular Docking

This gives the overall 3D structure, and the PyRx (https://pyrx.sourceforge.io/) helps us visualize the structures of the selected compounds.

RESULTS

Extraction and Purification of Target

We were able to download the 7L1X protein from the PDB format, as a PDB file.

Figure 1 shows the cleaned 7L1X protein, as it was visualized on the Discovery Studio BIOVIA app.

Structure Validation

Secondary Structure Validation

A group of bioinformatics techniques that use knowledge of a protein's or nucleic acid sequence's primary structure to predict the secondary structures of those structures.

In the case of proteins, protein structure formation like alpha helices and beta strands, are predicted.

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This is the secondary structure of the 7L1X protein that shows the Alpha Strand and Beta Sheet (Figure 2). It consists of 3 sheets, 4 beta hairpins, 5 beta bulges, 9 strands, 21 helices, 20 helix–helix interactions, 24 beta turns, and 5 gamma turns.

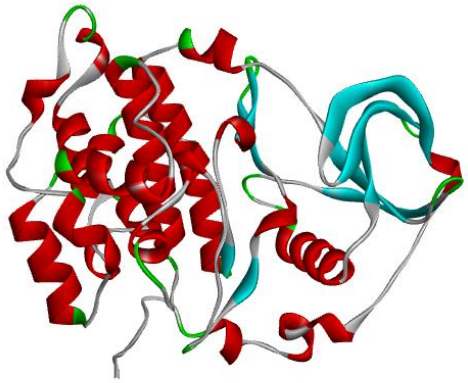

Figure 1. 3D purified structure of the 7L1X protein.

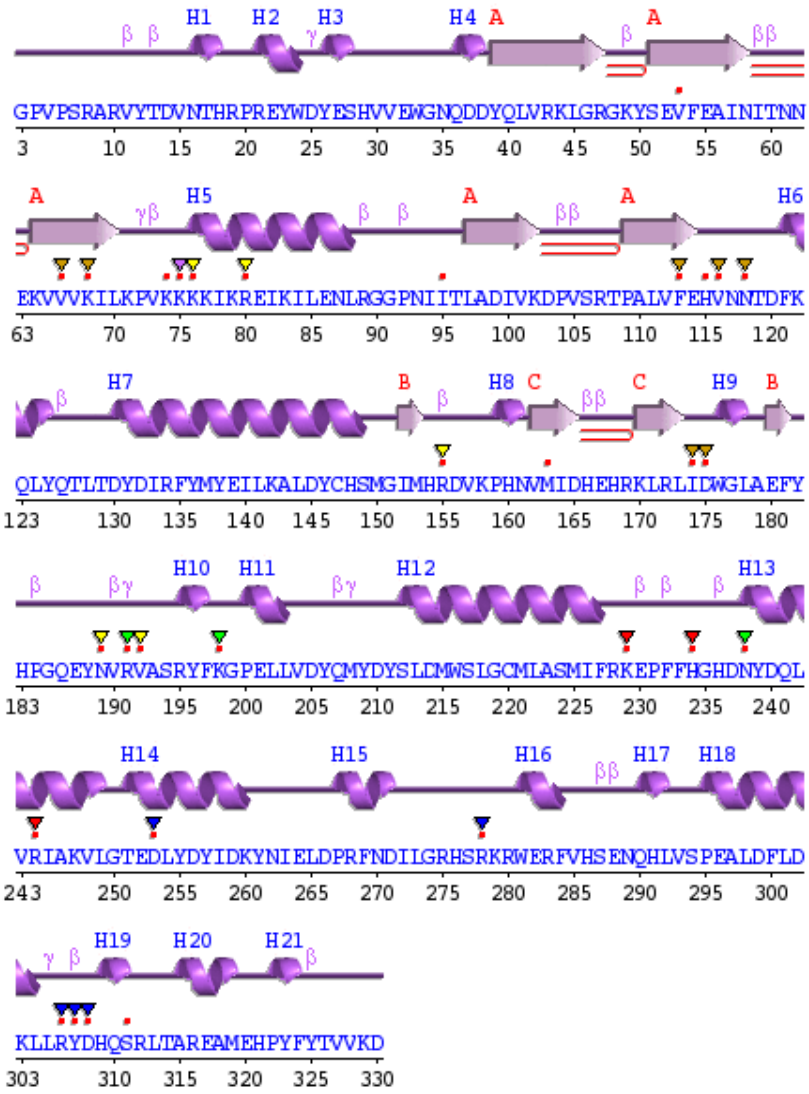

Figure 2. Secondary structure prediction.

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Ramachandran Plot

The Ramachandran Plot (Figure 3) consists of 601 most favored residues, which account for 93.9% of the total 640 non-glycine and non-proline residues. In the remaining residues, 35 are additionally allowed regions (5.5%), generously allowed regions is 1, accounting for 0.2%, and 3 are disallowed regions, accounting for 0.5%.

The score of the Psi-Phi distribution is 0.16, Chi1-chi2 distribution is –0.10, Chi1 only is 0.07, Chi3-Chi4 is 0.50, and Omega is –0.47, giving it an overall average of –0.06.

When it comes to the chain-chain covalent forces, the main chain bond length is 0.59 Å, while the main-chain bond angles are 0.31 Å, making it an average of 0.43 Å . The overall average is 0.14 Å.

A good quality model should have more than 90% in the most favored regions [A, B, L] according to an examination of 118 structures with a resolution of at least 2Å and an R-factor of no more than 20.0.

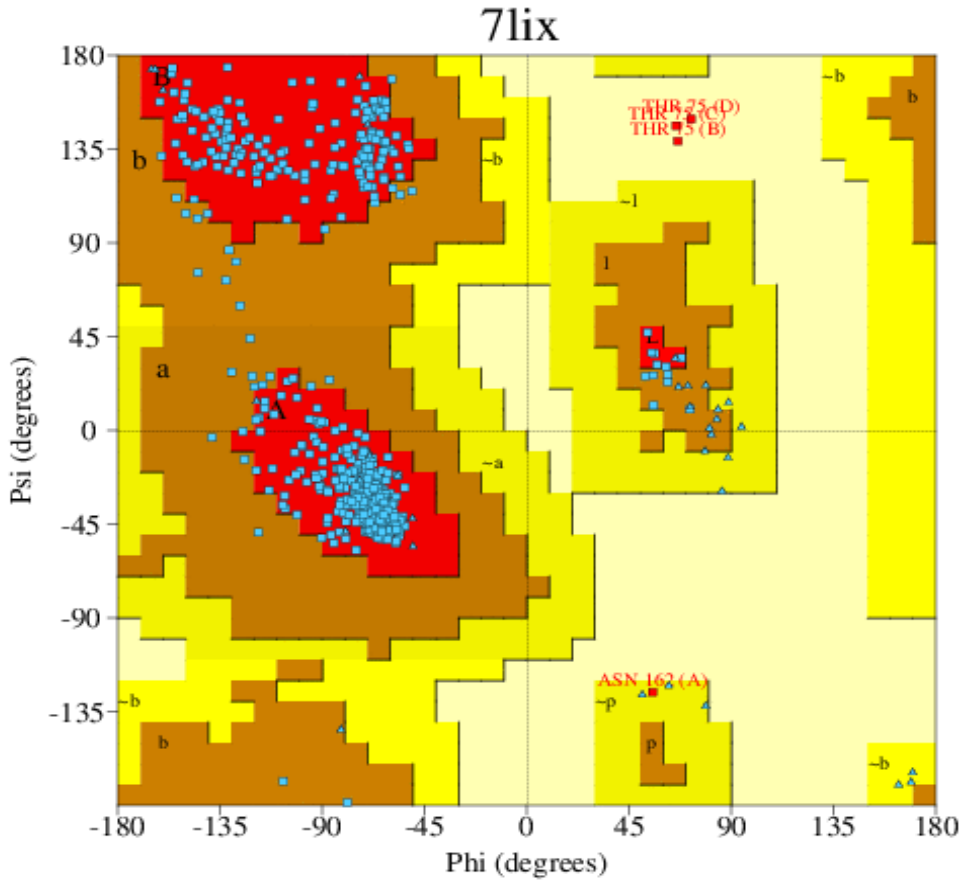

Figure 3. Ramachandran plot.

Retrieval of Ligands

Selected Phytocompounds from the IMPPAT Database

We select the plant "*G. biloba*" from which we take 20 phytocompounds. These are screened through IMPPAT database and their 3D structures analyzed through PubChem. We obtained the phytocompounds as shown in Table 1.

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Table 1. Extracted phytochemical compounds.

S.N.	Chemical Name
1.	Tannic acid
2.	Gallotannin
3.	Afzelin
4.	4-Hydroxycinnamic acid
5.	B-Sitosterol
6.	Stigmasterol
7	Kaempferol-7-O-glucoside
8.	Bilobol
9.	Zeatin riboside
10.	D-glucuronic acid
11.	Kaempferol
12.	Ginkgetin
13.	Acacetin
14.	Quercetin
15.	Apigenin
16.	B-D-xylopyranose
17.	Bilobetin
18.	Isoginkgetin
19.	Genkwanin
20.	Morin

ADME Analysis

We use the Swiss ADME website to do the ADME analysis. We find that the below compounds satisfy the conditions: the BBB Value should be low, GI absorption should be low, and Lipinski's rule should be satisfied and have 0 violations (Figure 4).

Interpretation

The structure on the top shows the parameters of the phytochemical compounds; the PGP+ value and the PGP- value. From there, we have selected the phytochemical compounds, which will be useful to treat TNBC which are summarized below (Table 2).

Table 2. Selected phytochemical compounds that satisfy the Lipinski's rule.

S.N.	Name	Formula	Molecular Weight (g/mol)	H Bond Acceptors	H Bond Donors	GI Absorption	Lipinski Rule (Violation)
1.	Afzelin	$C_9H_8O_3$	164.61	3	2	High	0
2.	Glucuronic acid	$C_6H_{10}O_7$	194.14	7	5	Low	0
3.	Ginkgetin	$C_6H_{12}O_5$	284.26	5	2	High	0
4.	Acacetin	$C_{15}H_{10}O_7$	302.24	7	5	High	0
5.	Quercetin	$C_{15}H_{10}O_5$	270.24	5	3	High	0
6.	Isoginkgetin	$C_6H_{12}O_5$	284.26	5	2	High	0
7.	Genkwanin	$C_{15}H_{10}O_7$	302.24 g/mol	7	5	High	0

Molecular Docking

After the docking in PyRx, we can visualize ligand interaction in Discovery Studio 2021 v21.1.0.20298 (BIOVIA). The 2D ligand interactions show the amino acids of the macromolecule by the van der Waals, conventional hydrogen bond, carbon–hydrogen bond, pi-donor hydrogen bond, pi-sigma, pi-pi stacked, pi-alkyl, alkyl, and unfavorable donor–donor with the specific color.

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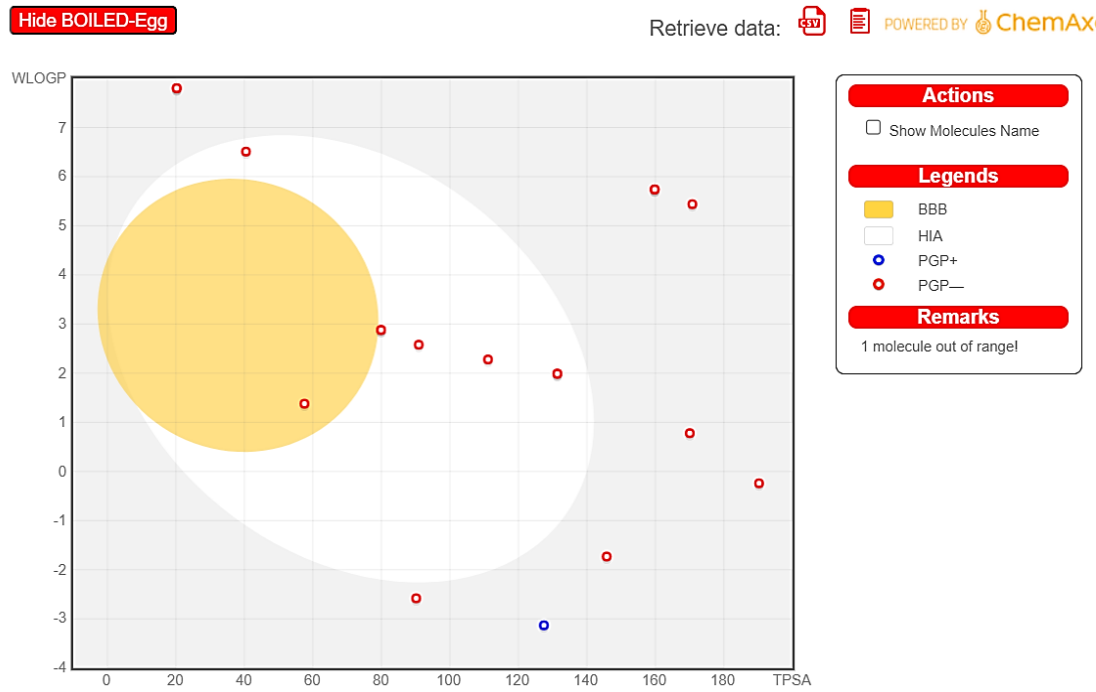

Figure 4. Boiled egg analysis.

We can find the name of the amino acid with position number and protein chain, the ligand-amino acid distance, and its type for docking interactions of the compounds Afzelin, D-Glucuronic acid, Gingketin, Acacetin, Quercetin, Isoginkgetin, and Genkwanin were interpreted from the 2D analysis were done as shown in Figure 5 with their binding affinity as shown in Table 3. The unfavorable interactional molecular docking of compounds is not a good inhibitor. The protein flexibility not taken, unexpected binding site or mode of action, solvent, entropy, and target interaction leads poorly accounted for during docking.

Table 3. Binding affinities.

Ligand	Binding Affinity
Cleaned-structure-7l1X_94715_uff_E=173.12	–5.6
Cleaned-structure-711X_5271805_uff_E=721.84	–10.4
Cleaned-structure-711X_5280343_uff_E=380.43	9.6
Cleaned-structure-711X_280442_uff_E=247.39	–9.4
Cleaned-structure-711X_5281617_uff_E=247.18	–9.6
Cleaned-structure-711X_5316673_uff_E=581.17	–9.4
Cleaned-structure-711X_5318569_uff_E=722.51	–10

DISCUSSION

HEGFR-2, progesterone, and estrogen are not present in TNBC. Over 15–20% of the breast cancers are caused by this cancer, which is particularly interesting because it is extremely invasive, hard to treat, and responds poorly to treatment.

TNBC accounts for 15–25% of all breast cancer cases. Every age group's TNBC proportion showed a similar pattern. On the other hand, basal TNBC, apocrine and neuroendocrine TNBC, and BRCA are more invasive in younger and older women. African Americans have a poorer prognosis than other ethnicities, and African American and Hispanic women are reported to be at a greater risk of TNBC. A 2009 case study found that 187 TNBC patients who had taken oral contraceptives for

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longer than a year had a 2.5% increased risk of getting TNBC. Women under 40 years of age have a 4.2% risk [16].

We first downloaded the files of the 7L1X protein from the PDB database. This is carried out in the PDB format. Secondary structure validation enables the identification of the positions of different structural components, including beta sheets, beta hairpins, beta bulges, strands, helices, helix–helix interactions, beta turns, and gamma turns.

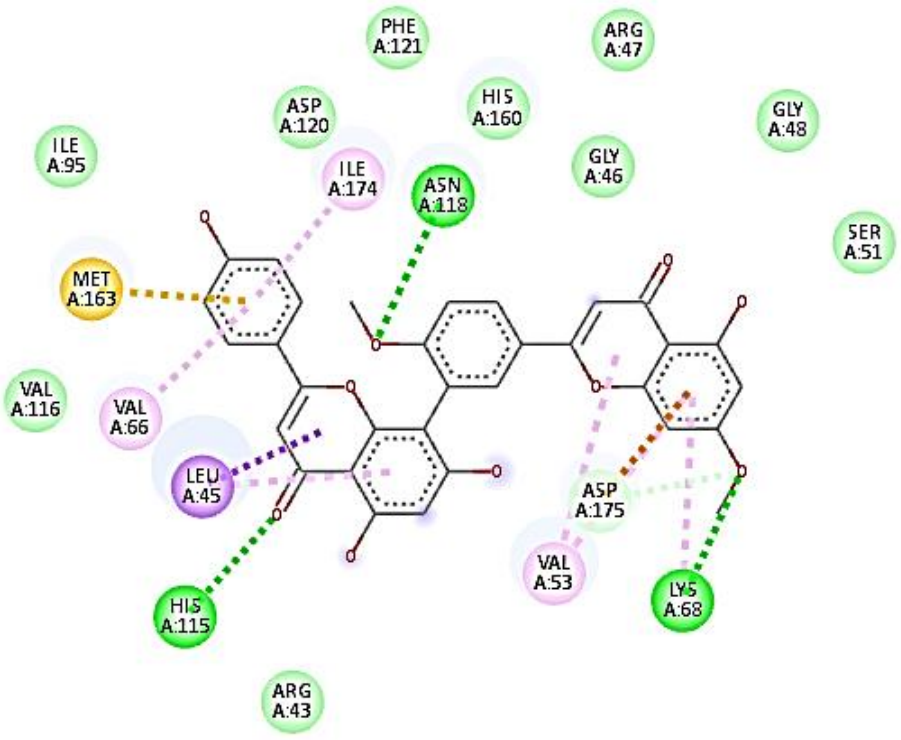

Figure 5. The visualized 2D structure of the ligand interactions.

The 2D representation of the φ-ψ torsion angles of the protein backbone is known as the Ramachandran Plot. It offers a basic perspective on a protein's structure. In the Ramachandran plot, the φ-ψ angles cluster into several regions, each of which corresponds to a different secondary structure. Depending on the stereochemistry of the amino acid, there are four fundamental types of Ramachandran plots: pre-proline (which refers to residues that come before a proline), glycine, proline, and generic (which refers to the 18 non-glycine non-proline amino acids [17].

The Ramachandran Plot helps us visualize the torsional angles of the protein; the favorable and unfavorable regions, and the G-factors. In our case, there are 605 out of 640 overall favorable regions, making it 93.9%, making the protein stable. Then, the phytochemical compounds are extracted from the IMPPAT database; 20 in all. ADME analysis enables us to find the compounds that satisfy Lipinski's rule, and the other conditions. Finally, we proceed with the molecular docking.

Molecular Docking is an essential technique in computer-aided drug design and structural molecular biology. The objective of ligand-protein docking is to predict the primary binding mode(s) of a ligand to a protein with a known 3D structure. Effective docking techniques employ a scoring system that accurately evaluates candidate dockings and efficiently explores high-dimensional spaces. Docking serves as a valuable tool for lead optimization by enabling virtual screening of large compound libraries, ranking the outcomes, and offering structural insights into how ligands interact

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with and potentially inhibit the target. However, interpreting the results from stochastic search methods can be challenging, and ensuring the proper configuration of input structures for the docking process is just as crucial as the docking procedure itself [18, 19].

In this step, we get the molecular docking values of the seven phytocompounds, which satisfy Lipinski's rule and the other conditions also. We got the cleaned ligand structure for all seven phytochemical compounds, and the molecular docking score also for all of them. The binding affinity scores for the seven compounds respectively are –5.6, –10.4, 9.6, –9.4, –9.6, –9.4, and –10. The lower the binding affinity score is, the more effective it is, i.e., the higher the affinity is. Therefore, the phytochemical compound Isoginkgetin, has the highest affinity for binding, with a score of –10.4.

We can also visualize the 2D structure of the ligand interactions, the above structure visualization (Figure 5). In the above structure, the light green structures are the van der Waals force of attraction. The dark green structures indicate the conventional H bonds between the various ligands.

CONCLUSIONS

Thus, the *G. biloba* plant in the treatment of the disease – TNBC can be used. The above-mentioned phytocompounds which satisfy Lipinski's rule and other properties are useful and can be utilized in the treatment of the disease. The compound isoginkgetin satisfy the Lipinski's rule with 0 violations, The GI absorption value should be high for the drug to have its proper effect, the drug should not permeate the brain blood barrier, as it may then affect the brain, the PGP substrate should be low, and the Lipinski's rule should be satisfied with 0 violations with biding affinity –10.4.

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Abbreviations

ADMET: Adsorption, Distribution, Metabolism, Excretion, Toxicity
BBB: Blood Brain Barrier
BRCA-1: Breast Cancer Gene-1
ER: Estrogen Receptor
FDA: Food and Drug Administration
HEGFR-2: Human Epidermal Growth Factor Receptor-2
IMPPAT: Indian Medicinal Plants, Phytochemistry and Therapeutics
PDB: Protein Database
PR: Progesterone Receptor
TNBC: Triple Negative Breast Cancer

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