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# FUNGAL SECONDARY METABOLITES: EMERGING THERAPEUTIC AGENTS FOR BREAST CANCER: IN-SILICO STUDY

**Shahad S. Alsharif [1] and Bayan H. Sajer [2, *]**

[1] Department of Biological Sciences, King Abdulaziz University, Jeddah 80200, Saudi Arabia.

[2] Immunology Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

**Correspondence: Bayan H. Sajer*

## ABSTRACT

Cancer is a prevalent and deadly disease that significantly impacts human health and well-being, ranking as a leading cause of death globally. Genetic variations or mutations in genes associated with breast cancer, such as BRCA1 and BRCA2, can result in the overexpression of tumor suppressor genes. Despite advancements in cancer research and medicine, the development of efficient and safe drugs for breast cancer remains a significant challenge due to the severe side effects associated with many existing treatments. Medicinal fungi have traditionally been used to treat various diseases. In this study, we aimed to investigate the effectiveness of secondary metabolites from *Aspergillus flavus* in targeting tumor suppressor proteins associated with breast cancer. We utilized an in-silico method to identify potential compounds that could act as strong inhibitors for breast cancer treatment. Molecular docking studies were conducted using compounds known to be effective against BRCA1, BRCA2, and TP53. The pharmacological profile of the secondary metabolite, including PASS, bioactivity scores, ADMET, cell line cytotoxicity, cardiac toxicity, and organ and endpoint toxicity, was comprehensively evaluated. Among the secondary metabolites analyzed, vitexin exhibited the highest binding affinity energy to BRCA1, BRCA2, and TP53 proteins, respectively. This discovery suggests that vitexin could be a promising candidate for the development of selective and potent tumor suppressor inhibitors for breast cancer treatment. This study represents an initial step towards the rational design of novel inhibitors for breast cancer therapy. By targeting tumor suppressor proteins such as TP53, BRCA1, and BRCA2, we aim to develop more effective and safer drugs for breast cancer patients.

**Keywords**: Molecular docking; pharmacological profile; breast cancer; tumor suppressor genes; drug; natural source.

## 1.Introduction

Breast cancer (BC) has become globally the utmost frequent cancer, surpassing all other malignancies [1]. In Saudi Arabia, it is also the most prevailing cancer, with a significant increase in incidence over the past 15 years. It ranks as the second primary source of mortality in the country [2-4]. According to data from the Saudi Cancer Registry, BC described for 14.2% of all cancer states and 29% of cases in Saudi female in 2020 [5].

The ASR (age-standardized prevalence rate) for the female population was 28.8 per 100,000 [6]. Most BCs are sporadic (90-95%), while 5-10% can be accredited by genetic susceptibility, particularly in patients with a robust family history of the disorder [7, 8]. Hereditary breast cancers, which make up a large portion (about 80-90%) of cases, are associated with germline mutations in the breast tumor suppressor gene 1 (BRCA1), Breast tumor suppressor gene 2 (BRCA2), and Tumor protein-53 (TP53) genes [9]. BRCA2 and BRCA1 are human tumor suppressor genes responsible for regulating DNA repair, transcriptional activation, apoptosis, cell-cycle checkpoint regulator, and chromosomal re-modeling [10, 11]. When a BRCA1 or BRCA2 mutation occurs, DNA repair is impaired, leading to an raised threat of breast cancer [12, 13]. Currently, BRCA2 and BRCA1 are considered the most significant "high-risk" genes, accounting for numerous issues of breast and ovarian cancer within families [14]. The TP53 gene functions as a tumor suppressor by regulating cell growth and division, safeguarding cells from DNA damage-induced genome alterations by inhibiting proliferation or inducing apoptosis [15]. The search for innovative and therapeutically effective anticancer drugs is driven by various motivating factors [16, 17]. Previous studies have highlighted the potential of various organisms, including plants, animals, insects, fungi, bacteria, and protozoans, to produce bioactive pharmaceutical compounds that can impact cancer cells [17, 18].

Fungi have a remarkable capacity to generate a wide range of useful compounds, making them unique among living organisms. *Aspergillus flavus* is known for producing a variety of bioactive compounds, including anticancer agents [19]. Among the secondary metabolites isolated from the endophytic fungi A. flavus, certain molecules such as vitexin, ergonovine, friedlein, orientin, beta-carotene, triterpenoids, homoorientin, luteolin, ergometrinine, phytosterols, and others have been found to have therapeutic benefits [20, 21]. The antimicrobial and antioxidant activities of A. flavus are attributed to its rich in their phenolics and flavonoid contents [21]. Fungi are valued for their pharmacological potential because of their rapid growth, adaptability to various conditions, high cell density, and genetic manipulability. They have produced approximately 148,000 natural chemicals, making them a promising source of medicinal drugs. Fungal metabolites consist of various compounds like amino acids, aromatic compounds, pyrones, butenolides, anthracenones, cytochalasans, macrolides, naphthalenes, and terpenes. It is worth noting that about 60% of existing anticancer treatments are isolated from natural resources [17].

Recently, the computational methods approach has gained popularity for identifying novel compounds against specific biologically active macromolecules. This approach includes various techniques such as evaluating biological activity, bioactivity scores, cell line cytotoxicity, cardiac toxicity, and organ and endpoint toxicity [19]. These approaches are widely employed in the

discovery, development, and analysis of drugs and other biologically active molecules. Molecular docking is a crucial computational modeling technique used to determine the preferred orientation of one molecule (known as the ligand) with another (known as the receptor) when they interact to form a stable complex [19, 22]. Docking offers several advantages over other computational molecular dynamics methods. This method allows for the prediction of optimized molecule or ligand orientation on a target, enabling the prediction of different binding modes[22]. Additionally, docking aids in the development of potent, selective, and efficient analogs. Molecular docking and scoring functions also facilitate the efficient in silico screening of large databases for potent drug candidates. This is particularly valuable in the field of chemotherapy, where understanding the cytotoxic mechanisms of drugs is crucial [23]. Researchers are currently investigating molecular docking to gain insights into how drugs bind to nucleic acids. This knowledge can then be used to design more effective drugs with fewer side effects. Overall, docking is a valuable tool in computational molecular dynamics, offering numerous benefits in drug discovery and development [24]. Our study intended to explore the potential effectiveness of fungal secondary metabolites, previously reported as inhibitors against human cancer, in targeting different proteins associated with BC, and if these metabolites could demonstrate efficacy with minimal or no side effects. To achieve this, several computational methods were utilized.

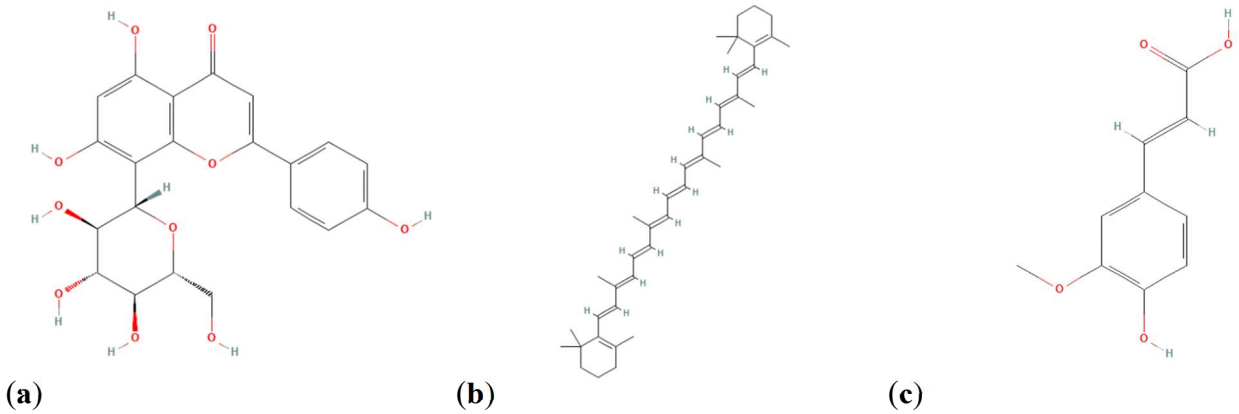
## 2. Materials and Methods

The computational software applied for this experiment contains AutoDockTools (v. 1.5.7), Discovery Studio (v.21.1.0.0), and other online computational devices accessible. This work did not require ethical approval as all data used in this study were extracted from websites. The selection of three secondary metabolites from the fungus *A. flavus* was reported in a recently published literature against breast cancer. The secondary metabolites selected include vitexin, beta-carotene, and ferulic acid.

### *2.1. Selection and Preparation of Ligands*

Three secondary metabolites from a fungal source were reported in a previous study as highly effective compounds with anticancer potential [19]. Secondary metabolites were collected and downloaded from a public database (https://pubchem.ncbi.nlm.nih.gov) in SDF format **Figure 1**. The chemical structure of the screened compounds used in this stud; (Fig. 1a) Vitexin (Fig. 1b) Beta-caroten (Fig. 1c) Ferulic acid

The ligands were converted to PDB format using the software Open Babel (http://openbabel.org/wiki/Main_Page) to prepare them for docking exploration. Torsion requirements for correct binding were defined using Autodocktool 4.2.6 variables [25], and the internal energies of the ligands were enhanced [26].

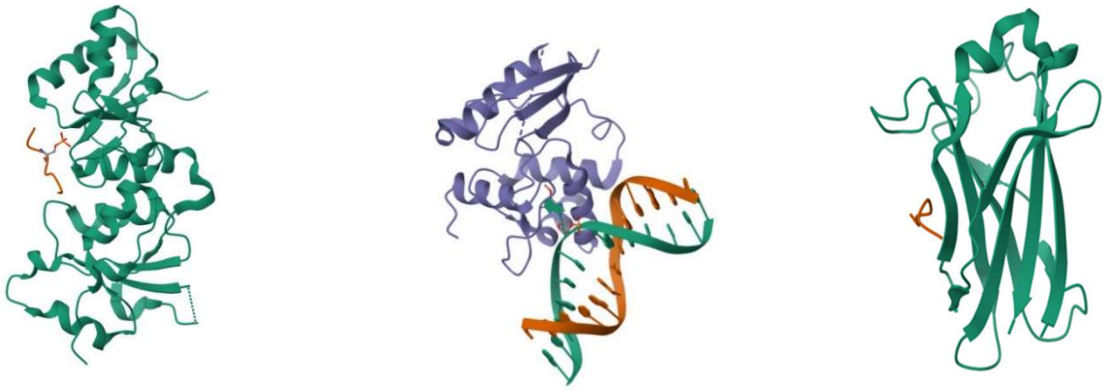


(a) (b) (c)

**Figure 1.** The chemical structure of the screened bioactive molecules used in this study; (a) Vitexin (b) Beta-carotene (c) Ferulic acid.

### *2.2. Proteins Collection and Preparation*

The 3D structures of proteins (Figure 2), including BRCA1 (Fig. 2a), BRCA2 (Fig. 2b), and TP53 (Fig. 2c), were collected and downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) with PDB ID 3PXE for BRCA1, 1T38 for BRCA2, and 7LIO for TP53. To prepare the proteins for docking, AutoDockTools (v.1.5.7) was used. Water fragments were removed, polar hydrogen was added, and Kollman's charges were applied before docking [25]. Both the drug molecules and protein structures applied for the exploration of docking conseqeunces were converted to PDBQT format [26].



(a) (b) (c)

**Figure 2.** The three-dimensional structure of (a) BRCA1 (3PXE), (b) BRCA2 (1T38), and (c) TP53(7LIO).

### *2.3. Molecular docking study*

In silico studies were conducted by preparing the proteins and ligands, followed by molecular docking using AutoDockTools. AutoDockTools is a valuable tool for predicting the

pharmacodynamics shape of drug candidates by scoring and orienting them to the receptor binding spots [27]. The docking results uncover the extent of ligand interaction with the active spot of the targeted protein, specifying the extent of ligand interaction with the desired protein's active site. The active sites, which are the coordinates of the target protein-ligand, were examined using Discovery Studio (v.21.1.0.0) and AutoDockTools (v. 1.5.7). To facilitate binding, specific dimensions for x, y, and z were set, and the grid box was centered to achieve favorable docking conformations. The grid file was saved as a (.gpf) file, and autogrid was executed. Docking calculations were performed using the Lamarckian genetic algorithm with the default of 10 runs. The dock file was saved as dpf, and after running AutoDock, final docked results were obtained in a (.dlg) file, providing evidence such as inhibition constant binding energy, and binding residues. The structures revealing the interaction between proteins and ligands as well as they were visualized using Discovery Studio [25]. The accuracy of the docking protocol was authorized by re-docking (self-docking) the bioactive molecules with the protein employed throughout the research.

### *2.4. Investigation of the Compounds for Lipinski's Rule of Five (RO5)*

Lipinski's Rule of Five (RO5) is utilized to assess the drug-likeness of a compound. The molecular actions and drug-likeness of the biological molecules were analyzed based on RO5. This rule, developed by Christopher A. Lipinski in 1997, serves as a guideline for evaluating drug likeness by considering parameters such as molecular weight, lipophilicity, hydrogen acceptor, hydrogen donor, and molar refractivity [28, 29]. In this work, ligands were showed for RO5 conformity using the Supercomputing facility for computational and bioinformatics biology (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

### *2.5. Physicochemical and ADMET properties*

The ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) and physicochemical analyses were conducted to determine the pharmacodynamic properties of the molecule. The ADMET evaluation of the chosen potential compounds was performed using the pkCSM platform and SwissADME (http://www.swissadme.ch/index.php). For physicochemical activities, only SwissADME was utilized. This assay aims to provide valuable insights for drug research and development [25].

The assessment was conducted for each physicochemical property and ADMET by presenting a SMILE format of the single molecule acquired from the PubChem database. The investigation was established on a comprehensive database containing 288,967 attempts from peer-reviewed publications, EPA, ChEMBL, and DrugBank databases. For ADMET properties, all the files were confirmed and handled using the Molecular Operating Environment (MOE, version 2016). The properties were divided into 6 modules (basic, A, D, M, E, and T) and a sequences of subclasses based on their endpoint implications [30].

Absorption variables: Caco2 cell permeability, P-glycoprotein inhibition, human intestinal absorption, water solubility in a buffer system, and skin permeability.

Distribution variables: blood-brain barrier (BBB), RO5, and central nervous system (CNS) permeability.
Excretion variables: renal OCT2 substrate and total renal clearance.
Toxicity variables: oral rat acute, Tetrahymena pyriformis toxicity, AMES test, chronic toxicity, hepatotoxicity, skin sensitization, and Minnow toxicity.
Metabolism variables: Cytochrome P450 (CYP)1A2, CYP2D6, CYP2C9, CYP2C19, and CYP2D6, CYP3A4 inhibition, and CYP3A4 substrate.

### *2.6. Bioactivity Score and Bioavailability Radar*

The bioactivity score of ligands was detected by applying the online Molinspiration software (http://www.molinspiration.com/). This approach was accomplished by inputting SDF files of ligands acquired from PubChem website. The properties analyzed included enzyme inhibitors (EI), nuclear receptor ligands (NRL), G-protein-coupled receptors (GPCR), kinase inhibitors (KI), and ion channel modulators (ICM). The bioavailability radar of ligands was established using Swiss ADME (http://www.swissadme.ch/index.php), which immediately indicates if a compound is orally bioavailable. Secondary metabolites with binding energy comparable to typical treatments underwent this exploration.

### *2.7. Assessment of Blockage of hERG K+ Channels*

The human ether-à-go-go-related gene (hERG) encodes the subunit of a voltage-gated K+ channel that acts as a delayed rectifier, crucial for cardiac action potential repolarization [31]. Dysfunction of the hERG gene [32], can lead to substantial delays in heartbeats, potentially resulting in sudden death [31].
Computational methods are increasingly used for rapid and cost-effective assessment of chemical compounds. The Pred-hERG 5.0 web server is a valuable tool for evaluating the toxicity of metabolites by assessing hERG K+ channel blockage. This web server utilizes advanced machine learning models to predict hERG blockage accurately, with an expanded database, meticulous data curation, diverse prediction models, and improved accuracy. It offers a user-friendly interface for drawing chemical structures, uploading compound files, initiating evaluations, and reviewing results. Pred-hERG 5.0 is essential for assessing compound cardiotoxicity, a requirement in US FDA clinical trials [33]. The results are stated as probability forecasts, distinguishing between compounds that block and those that do not block the hERG channel. A confidence value not exceeding 0.26 indicates a non-cardiotoxic compound [34]. The segments that depict hERG inhibition can be seen on the probability map.
We used the cardioTox CAM web server, a computational platform designed for assessing cardiotoxicity. This platform can accurately predict six common clinical cardiac toxicity outcomes: hERG toxicity, heart block, cardiac failure, myocardial infarction, arrhythmia, and hypertension. Furthermore, it can identify substructures that are supplemented in toxic constituents, delivering validation for explanation and further evaluation [35].

### *2.8. Prediction of Cell Line Cytotoxicity*

Cytotoxicity prediction is achieved using the freely available online tool CLC-Pred 2.0 (Cell Line Cytotoxicity Predictor) [36]. An accessible online tool called PASS CLC Pred has been developed

to forecast the cytotoxicity of chemicals against tumor and normal human cell lines from various tissues using the mentioned PASS models. (http://www.way2drug.com/Cell-line/) [37]. PASS has the capability to predict around 4000 different biological activities, including machineries of action, toxic and adverse properties, interactions with metabolic enzymes and transporters, as well as pharmacological consequences on transcriptomic analysis [38]. The molecular structures of Vitexin, beta-carotene, and Ferulic acid were submitted in SMILES format. The resulting data was shown as activation probability (Pa) and inactivation probability (Pi) values [39]. These values can range from 0.000 to 1.000 [40]. When Pa > Pi, a specific compound is deemed to exhibit potential activity. The pharmacological action is deemed strong when Pa > 0.7 and weak when 0.5 < Pa < 0.7 [41]. Data can be pulled out in a structured data file format. The analysis of data obtained from CLC-Pred is interpreted according to their IC50 (half maximal inhibitory concentration), IG50 (half-maximal inhibitory growth), and % inhibition (of activity) values. Molecules are deemed active if they exhibit inhibition greater than 50% with IG50 and IC50 values of 10,000 nM [42].

### *2.9. Toxicity profile predication*

Evaluating the toxicological characteristics of a medication is crucial before advancing to the clinical trial or pharmaceutical production stages and eventual approval [43]. By using pkCSM and GUSAR servers, we successfully predicted various toxicities, including environmental toxicity, such as Tetrahymena pyriformis and Minnow toxicity.

### *2.10. Estimation of Biological Action Spectra for Bio-Molecules by Using PASS Webserver Prediction*

Based on the structures using a multilevel description of atom neighbors, the PASS web server (http://www.way2drug.com/passonline/) can be utilized to calculate the biologic action of a compound. The SMILES of the biomolecules is taken as the input, and the possibility of biological action can be gotten as the end product [43].

### *2.11. Prediction of Organ and Endpoint Toxicity*

We utilized the ProTox-II web server to assess the potential toxicity of the identified metabolites (https://tox-new.charite.de/protox_II/). The ProTox-II web server offers numerous help over current computational simulations. It encompasses knowledge of both molecular and chemical targets.

Additionally, its prediction scheme categorizes toxicity into various levels, including hepatotoxicity, oral toxicity, toxicological screening (like carcinogenicity, immuno-toxicity, mutagenicity, and cytotoxicity), toxicological pathways (AOPs), and toxicity targets. This provides valuable awareness into the potential molecular mechanisms underlying toxic responses [44]. We utilized the SMILES format (Simplified Molecular-Input Line-Entry System) string of the molecules obtained from PubChem(https:/pubchem.ncbi.nlm.nih.gov/) to conduct chemical structure searches based on the compound name. This method played a pivotal role in guaranteeing the precision of our predictions for these molecules, delivering a thorough evaluation of their toxicity shapes [45].

## 3.Results and Discussion

### 3.1. Molecular Docking Analysis

Docking is fundamental to expect a robust virtually and binder monitor a record of molecules. Due to the roles of the tumor suppressor genes that play in the development of cancer when those genes are lost or inactivated, it is required to develop appropriate drug candidates that can efficiently hinder the growth of these tumors with little or no properties. Binding affinity between receptors and ligands is established by the lower the energy, the binding energy, the higher the binding affinity. For this target, three secondary metabolites were screened against BRCA1/3PXE, BRCA2/1T38, and TP53/7LIO. After docking, inhibitory constant (μM/nM) showed in (**Table 1**), the binding energy (kcal/mol), number of hydrogen bonds, and amino acids implied of these compounds were also noted.

**Table 1.** The results of estimated inhibition constant, Ki using an AutoDock tool software.

| **Protein** | **Ki of Functional group 1 (vitexin)** | **Ki of Functional group 2 (beta-carotene)** | **Ki of Functional group 3 (ferulic Acid)** |
|---|---|---|---|
| ***BRCA1* (3PXE)** | 109.07 nM | 870.08 nM | 4.70 uM |
| ***BRCA2* (1T38)** | 140.61 nM | 860.61 nM | 401.62 nM |
| ***TP53* (7LIO)** | 278.43 nM | 7.42 uM | 11.57 uM |

Ki, Estimated Inhibition Constant, uM=micromolar, nM= nanomolar

The secondary metabolites vitexin, beta-carotene, and Ferulic Acid displayed binding energies including -9.5, -8.27, and -7.27 (kcal/mol), respectively, against BRCA1 (3PXE). While vitexin, beta-carotene, and ferulic Acid against BRCA2 (1T38) exhibited binding energies including -9.35, -8.27, and -8.73 (kcal/mol). Vitexin, beta-carotene, and ferulic Acid showed binding energies including -8.94, -7.0, and -6.73, respectively, against TP53 (7LIO). In comparison with the other compounds, vitexin displayed the highest binding energy against all receptors (**Table 2**).

**Table 2.** The results of docking for some secondary metabolite against BRCA1, BRCA2, and TP53 proteins.

| **Compounds** | ***BRCA1* (3PXE)** | ***BRCA2* (1T38)** | ***TP53* (7LIO)** |
|---|---|---|---|
| **Vitexin** | -9.5 | -9.35 | -8.94* |
| **Beta-carotene** | -8.27 | -8.27 | -7.0 |
| **Ferulic acid** | -7.27 | -8.73 | -6.73 |

*The unit of binding energy = kcal/mol

### *3.2. Interaction and Binding Affinity of Compounds*

Herein, the interactions, hydrogen bonds, and residues of amino acids were calculated using Autodocktools 4.2.6 and Discovery Studio Software for generating a 2D structure. For the BRCA1 (3PXE) protein, vitexin showed the highest binding energy compared to the other derivatives, exhibiting a binding affinity of -9.5 kcal/mol (Table 3). The compound vitexin showed strong hydrogen bonding with Ala1693, Glu1735, and His1732, and exhibited one pi-pi bond with Phe1695, and Phe1734 (**Figure 3**). In addition, beta-carotene exhibited van der Waals interactions with Glu1754, Asp1757, Arg1751, Ser1755, Asp1757, Tyr1845, Gln1846, Lys1759, Arg1762, Ile1760, Ile1807, and Pro1806; for amino acids that participated in Alkyl bonds, they were Arg1758, Cys1847, Leu1764, Pro1831, His1805, and Leu1850 (**Figure 4 and Table 3**). The ferulic acid compound showed several van der Waals bonds with Phe1695, Asp1733, Phe1734, His1732, and Arg1753 (**Figure 5**), also, exhibited one carbon-hydrogen bond with Val1736, as well as Alkyl interactions with Lys1750 (**Table 3**). The 2D and 3D structures of molecular interactions are presented in **Figures 3-5.**

**Table 3.** Docking consequences for the best binding affinity with BRCA1(3PXE) protein for three selected molecules.

| **Drug** | **Binding affinity (kcal/mol)** | **No. of H-bond** | **Residues Amino Acids** |
|---|---|---|---|
| **Vitexin** | -9.5 | 4 | Ala1693, Phe1695, Phe1734, Asp1733, His1732, Glu1735 |
| **Beta-carotene** | -8.27 | 0 | Lys1759, Arg1762, Arg1758, Ser1755, Arg1751, Glu1754, Asp1757, Tyr1845, Gln1846, Ile1760, Cys1847, Ile1807, Leu1764, Pro1806, Leu1850, His1805, Pro1831 |
| **Ferulic acid** | -7.27 | 3 | Glu1694, Phe1695, Arg1737, Asp1733, Phe1734, His1732, Glu1735, Arg1753, Val1736, Gln1747, Lys1750 |

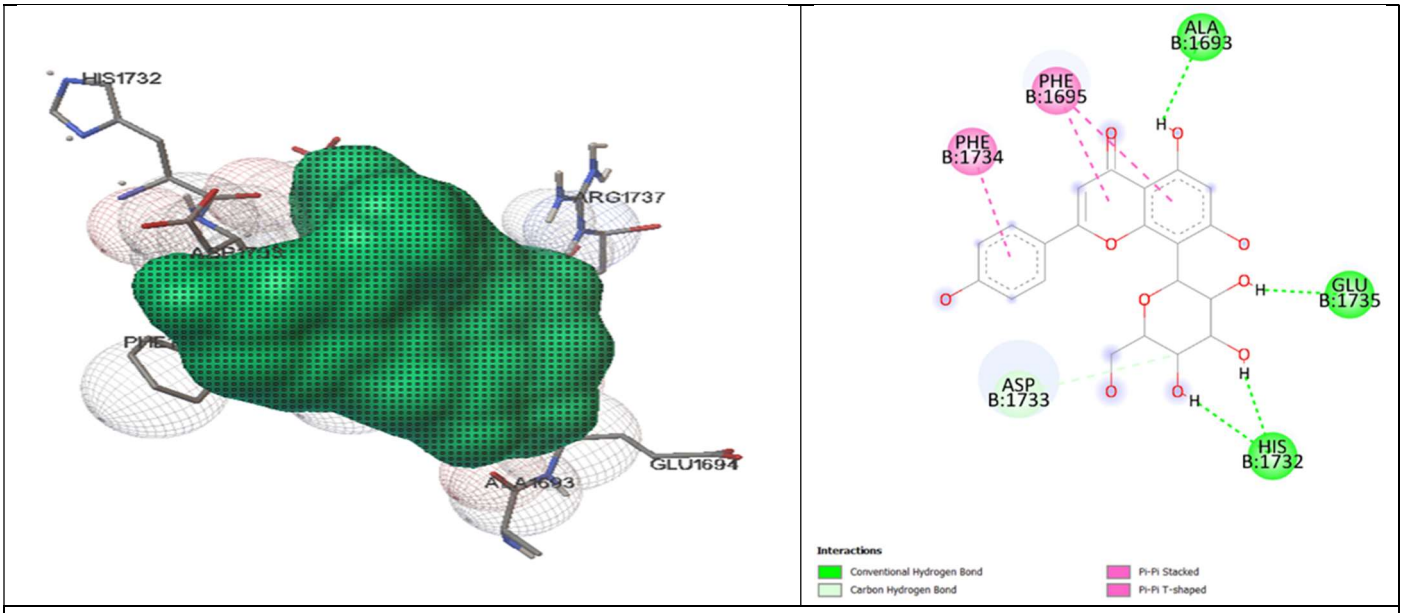



**Figure 3.** The molecular interaction *BRCA1* (3PXE) and Vitexin.

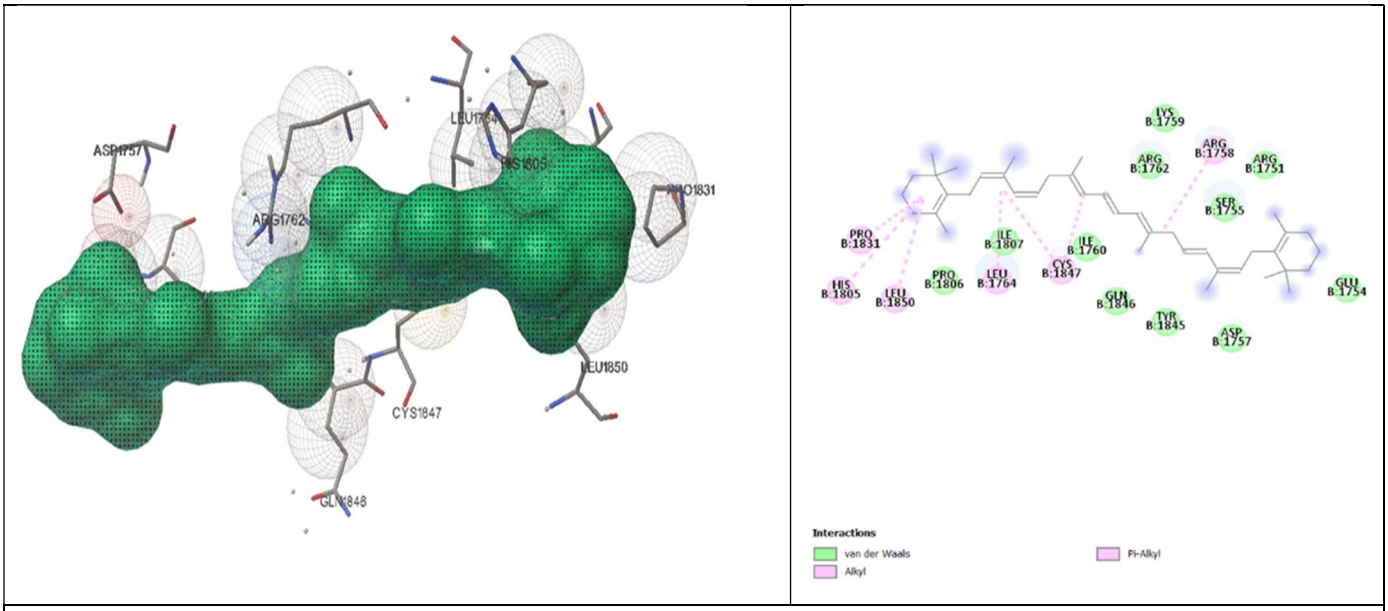



**Figure 4.** The molecular interaction *BRCA1* (3PXE) and beta-carotene.

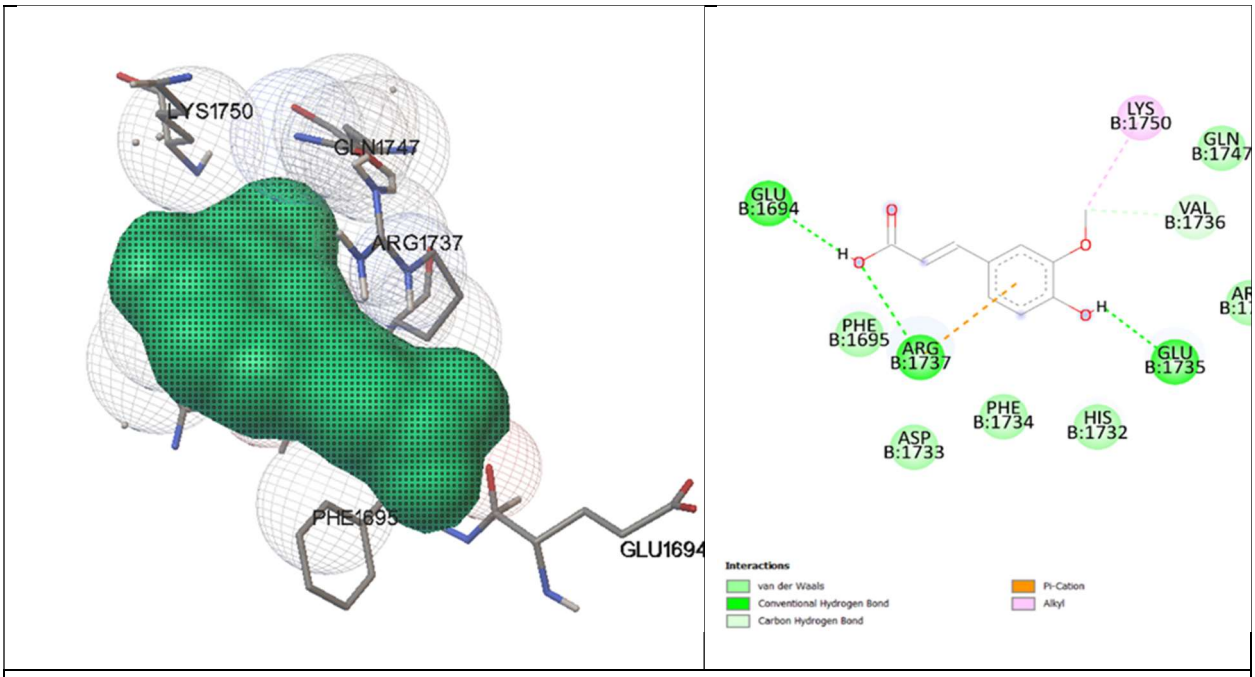



**Figure 5**. The molecular interaction *BRCA1* (3PXE) and Ferulic Acid.

### *3.3. Interaction and Binding Affinity of Compounds*

The compounds vitexin, beta-carotene, and ferulic acid showed high binding results towards the BRCA2 (1T38) protein, with binding energies of -9.35, -8.27, and -8.73 kcal/mol, respectively (**Table 4**). In the vitexin compound, we identified four strong hydrogen bonds with Ala83, Val81, Phe89, and Trp100 , as well as a Pi-Alkyl bond with Leu84 (**Figure 6**). Additionally, there were van der Waals interactions with Gln90, Arg96, Lys104, Lys101, Leu99, Leu103, and Pro82. Beta-carotene exhibited five hydrophobic interactions (alkyl and pi-alkyl bonds) with Trp65, Ile76, Kys107, Leu103, and Lys104, along with a Pi-sigma bond with Trp100 (**Figure 7**). Van der Waals interactions were observed with Pro144, Val106, Arg147, Phe108, Glu77, Val81, Kys101, Glu92, Gln97, and Arg96.

In Ferulic acid, three hydrogen bonds were identified with Leu33, Asn137, and Tyr114, as well as an unfavorable sump with Lys165 (**Figure 8**). Alkyl and pi-alkyl bonds were formed with Met134, and a Pi-sigma bond with Tyr158. Van der Waals interactions were observed with Pro140, Ser145, Ser159, Arg135, Ser145, Gly131, Asn157, and Val148. The 2D and 3D structures of molecular interactions are depicted in Figures 6-8.

**Table 4.** The results of docking analysis for best binding affinity with BRCA2 (1T38) protein for three selected biomolecules.

| **Drug** | **Binding affinity(kcal/mol)** | **No. of H-bond** | **Residues Amino Acids** |
|---|---|---|---|
| **Vitexin** | -9.35 | 4 | Ala83, Val81, Pro82, Leu84, Phe89, Gln90, Arg96, Lys104, Trp100, Lys101, Leu99, Leu103 |
| **Beta-carotene** | -8.27 | 0 | Glu92, Arp100, Pro144, Val106, Trp65, Ile76, Arg147, Lys107, Phe108, Glu77, Val81, Phe79, Leu103, Lys104, Lys101, Gln97, Arg96, Trp100. |
| **Ferulic acid** | -8.73 | 2 | Leu33, Pro140, Ser145, Val148, Lys165, Asn157, Tyr114, Gly131, Met134, Arg135, Tyr158, Ser159, Asn137 |

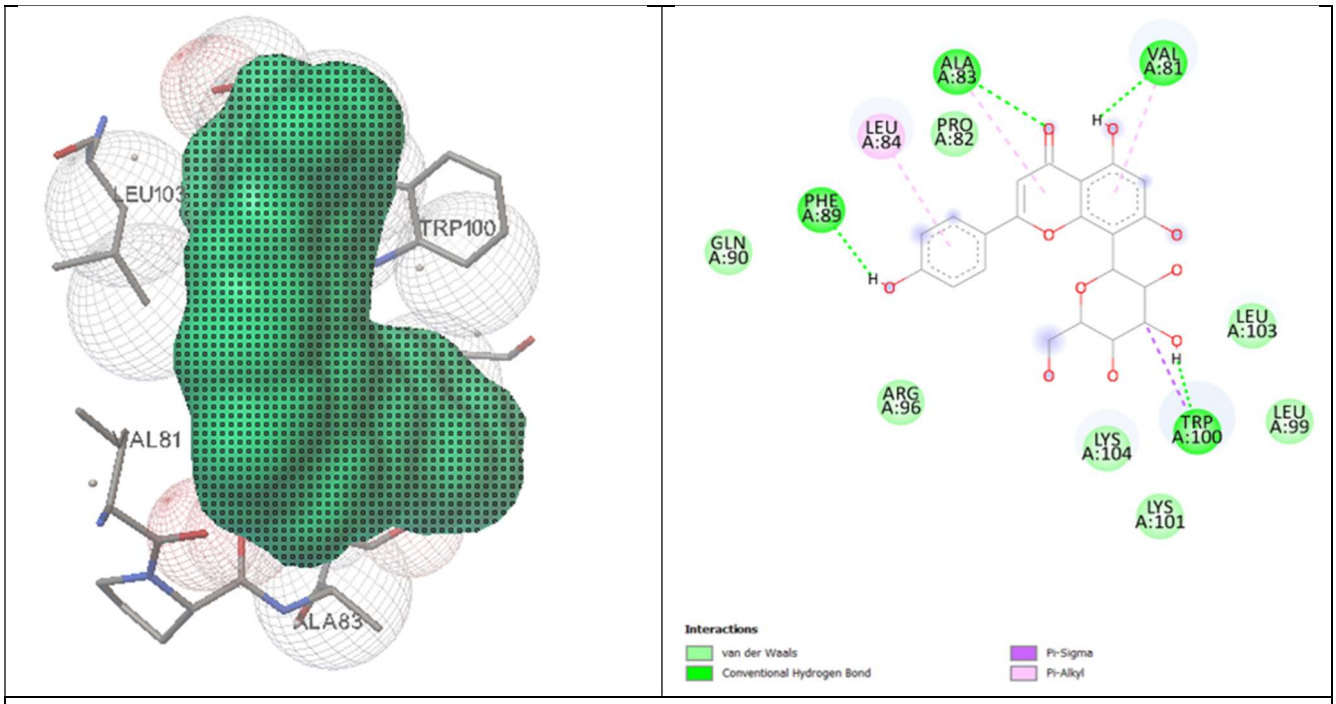



**Figure 6.** The molecular interaction *BRCA2* (1T38) and Vitexin.

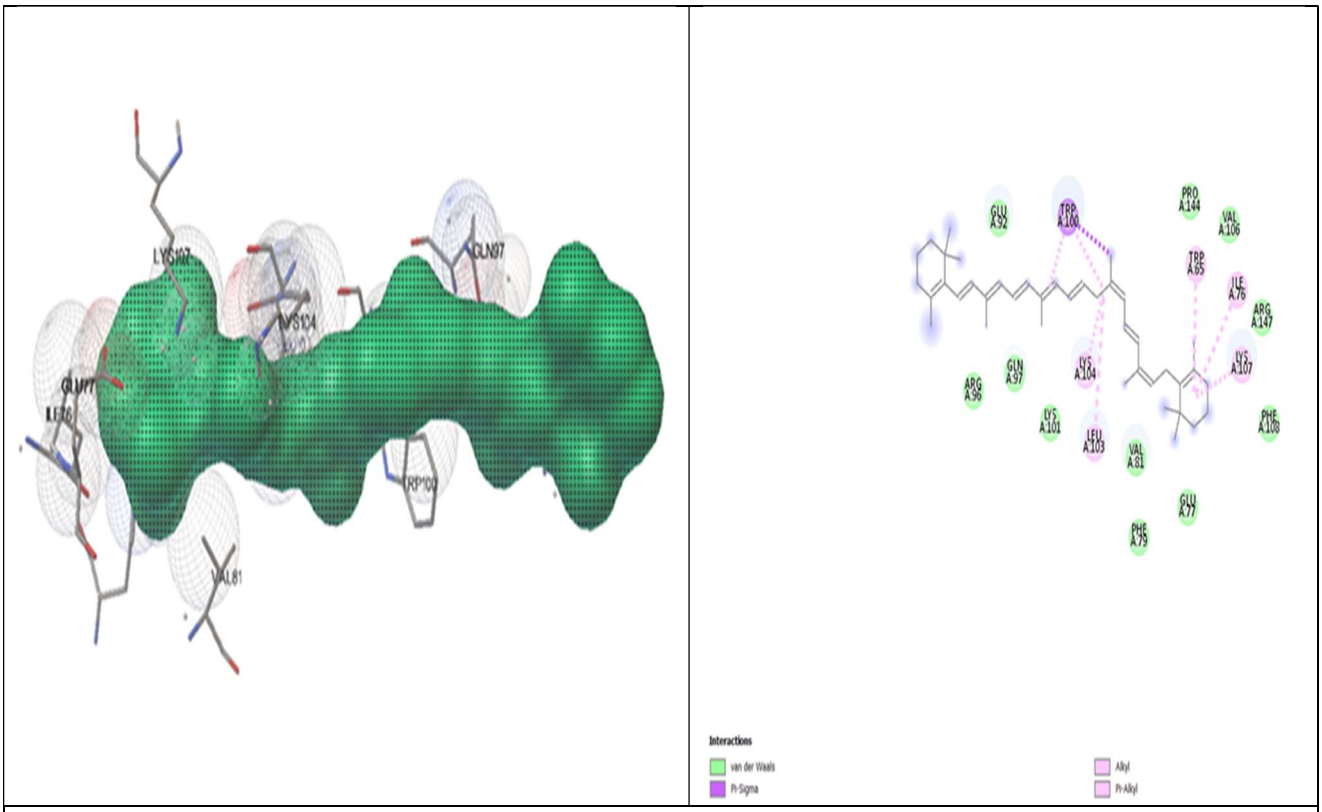



**Figure 7.** The molecular interaction *BRCA2* (1T38) and beta-carotene

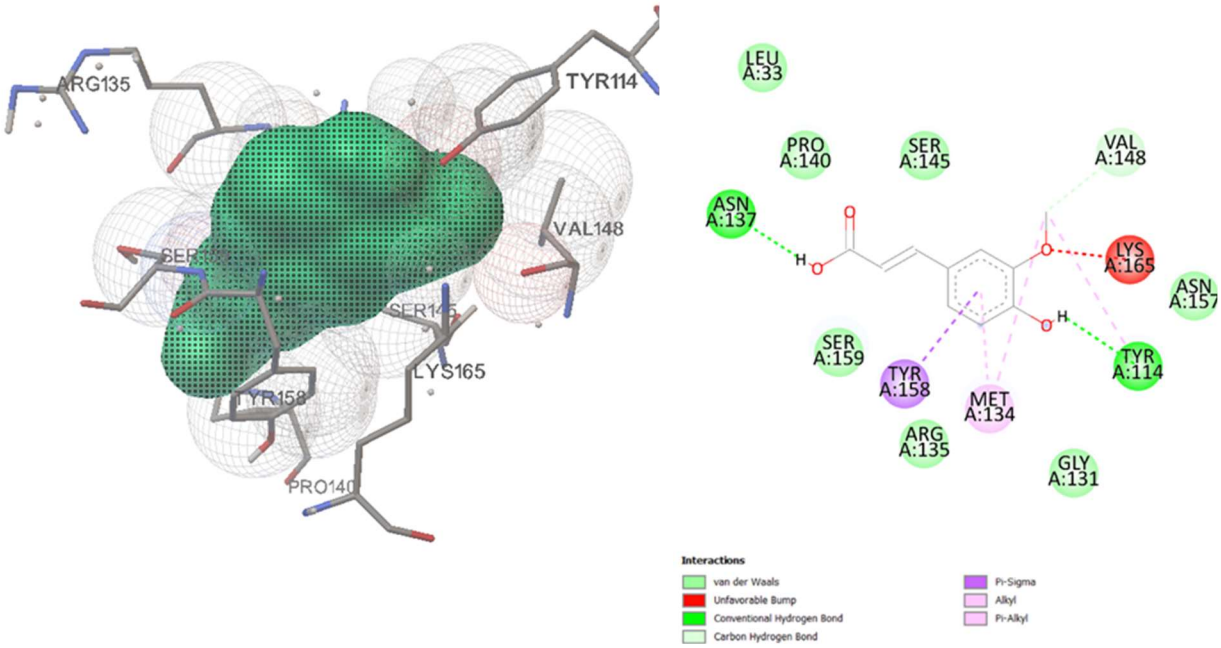



**Figure 8.** The molecular interaction *BRCA2* (1T38) and Ferulic Acid.

### *3.4. Interaction and Binding Affinity of Biomolecules*

Among the compounds tested, vitexin showed significantly improved binding energy towards the TP53 (7LIO) protein compared to the other compounds. Vitexin formed strong hydrogen bonds with Thr56, Ser33, and Ser58, as well as one Pi-Pi interaction with Tyr34 (**Figure 9, Table 5**). On the other hand, beta-carotene formed several Pi-alkyl interactions with Leu150, Ala109, Lys110, and one Pi-sigma interaction with Phe43 (**Figure 10**). Ferulic Acid exhibited a Carbon-hydrogen bond with Glu97 and Leu65, one Pi-sigma interaction with Val98, van der Waals interactions with Val30, Arg99, Arg124, and Ser59, and one hydrogen bond with Val164 (**Figure 11).** Additionally, there were Pi-alkyl interactions with Lys64, Pro94, Lys95, Val163, and Val163. The 2D and 3D structures of molecular interactions are presented in Figures 9-11.

**Table 5.** Docking results for best binding affinity with TP53 (7LIO) protein for three compounds.

| **Drug** | **Binding affinity(kcal/mol)** | **No. of H-bond** | **Residues Amino Acids** |
|---|---|---|---|
| **Vitexin** | -8.94 | 4 | Tyr34, Thr56, Ser33, Ser58 |

| | | | |
|---|---|---|---|
| **Beta-carotene** | -7.0 | 0 | Leu150, Ala109, Lys110, Phe43 |
| **Ferulic acid** | -6.73 | 2 | Val30, Trp67, Leu65, Lys64, Pro94, Ser59, Lys95, Val163, Val164, Arg99, Arg124, Glu97, Val98 |

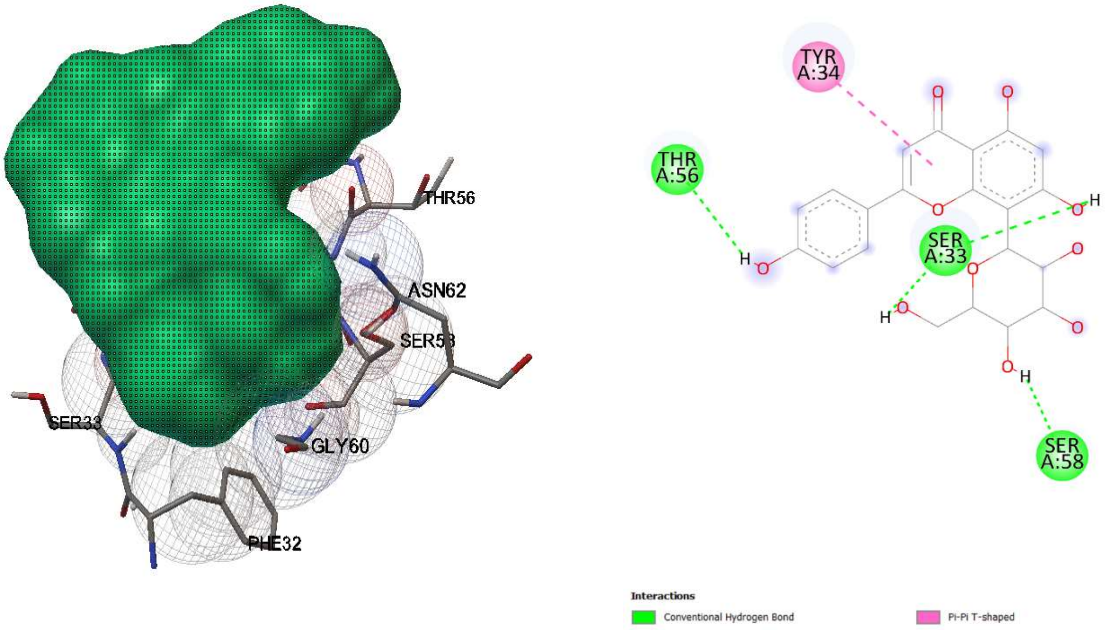



**Figure 9**. The molecular interaction *TP53* (7LIO) and Vitexin.

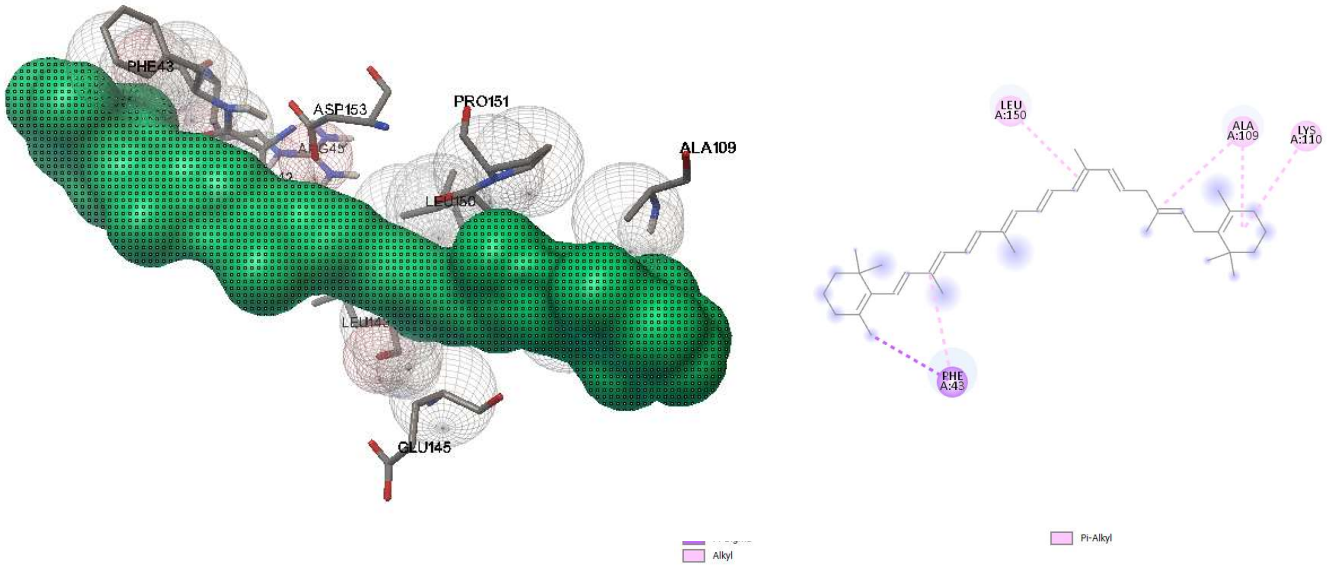



**Figure 10.** The molecular interaction *TP53* (7LIO) and beta-carotene.

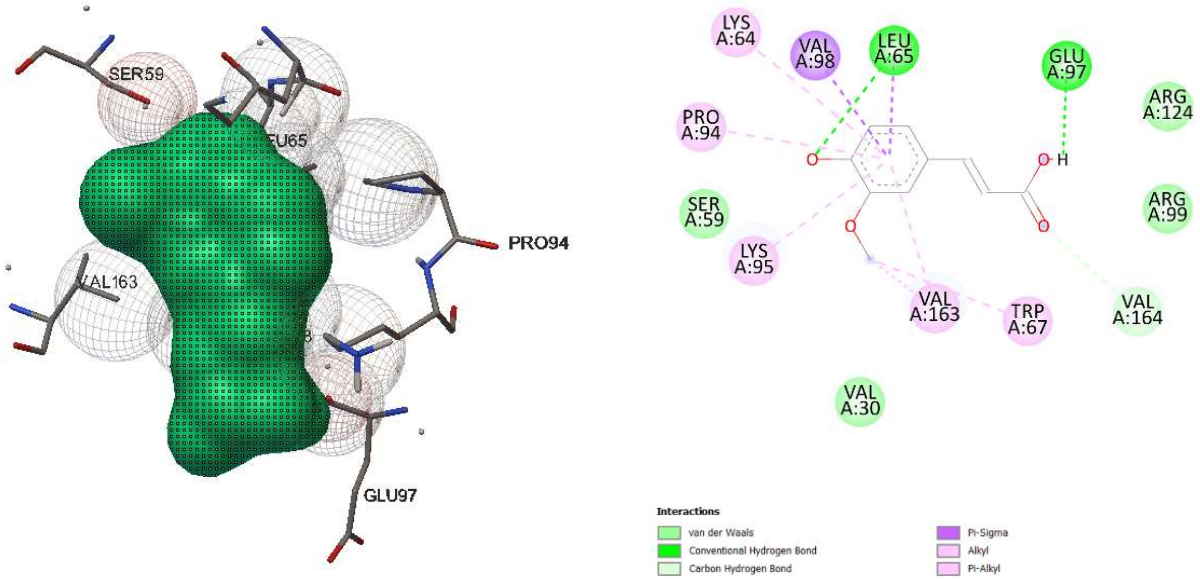



**Figure 11.** The molecular interaction *TP53* (7LIO) and Ferulic Acid.

After conducting multiple docking simulations, the interaction of the ligand with the protein was analyzed. It was found that among the secondary metabolites tested, vitexin and beta-carotene

exhibited the strongest binding interactions with all three proteins: BRCA1, BRCA2, and TP53. This discovery could be valuable for identifying and developing new preventive and therapeutic drugs for breast cancer.

### *3.5. Lipinski's Rule of Five (RO5) Analysis*

Lipinski's Rule of Five, also known as Pfizer's Rule, is a method used to assess the drug-like properties of a biological molecule. This rule helps determine if a chemical compound influences specific biological actions or pharmacological properties that could potentially make it an applicable oral drug in humans[46]. One crucial stage in drug discovery involves evaluating whether a particular compound is likely to be effective when taken orally. Lipinski's rule outlines the criteria for oral activity, stating that a drug should not violate more than one of the following conditions: molecular mass not exceeding 500 Daltons, a LogP value below 5 indicating limited lipophilicity, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a molar refractivity falling within the range of 40-130 [29, 47]. The data stated in **Table 6** show that Ferulic Acid satisfied all five Lipinski criteria, while beta-carotene did not meet the criteria. Vitexin, however, only deviated from one of the criteria, which is considered acceptable. Lipinski's rule suggests that an orally active drug should generally not violate more than one of the specified criteria.

**Table 6.** Lipinski's rule of 5, of the selected biomolecules used in this study.

| Secondary metabolite | Mass | Hydrogen bond donor | Hydrogen bond acceptor | LOGP | Molar refractivity |
|---|---|---|---|---|---|
| **Vitexin** | 432 | 7 | 10 | -0.065500 | 103.534050 |
| **Beta-carotene** | 536 | 0 | 0 | 12.605807 | 181.392334 |
| **Ferulic acid** | 194 | 2 | 4 | 1.498600 | 51.328594 |

### *3.6. Physicochemical and ADMET analysis*

After the drug is administered to the human body or an animal model via any route, it undergoes ADMET processes, leading to its active or passive transport to the target site [48]. When interacting with specific biological macromolecules, there can be both positive and negative pharmacological outcomes. The efficacy of a drug and its ability to reach the intended target in the body are influenced by its safety and efficacy. The primary reason for the failure of a drug is often its inadequate safety and efficacy, which is closely linked to its ADMET properties. In this study, the ADMET properties of three selected compounds were assessed using different in silico tools such as the SwissADME server, pkCSM server, and GUSAR. These evaluations aimed to gauge

the pharmacokinetic properties, including lipophilicity, water-solubility, drug-likeness, medicinal chemistry, and toxicity of the compounds[49]. The compounds' lipophilicity enables them to readily pass through the cell membrane, making oral preparation unsuitable. Furthermore, an injectable dosage form might be a more favorable choice to attain a swift onset of action due to low gastrointestinal absorption. The physicochemical and ADMET actions of the chosen compounds are detailed in **Tables 7 and 8.**

**Table 7**. Predicted lipophilicity and physicochemical variables of selected compounds generated using a SwissADME server.

| **Properties** | **Parameters** | **Vitexin** | **Beta-carotene** | **Ferulic acid** |
|---|---|---|---|---|
| **Physicochemical Properties** | MW (g/mol) | 432.38 | 536.87 | 194.18 |
| | Rotatable bonds | 3 | 10 | 3 |
| | HBA | 10 | 0 | 4 |
| | HBD | 7 | 0 | 2 |
| | Fraction Csp3 | 0.29 | 0.45 | 0.10 |
| | TPSA | 181.05 | 0 | 66.76 |
| **Lipophilicity Log *Po/w*** | iLOGP | 1.38 | 7.79 | 1.62 |
| | XLOGP3 | 0.21 | 13.54 | 1.51 |
| | MLOGP | -2.02 | 8.96 | 1.00 |
| | Consensus | -0.07 | 11.11 | 1.36 |

MW= Molecular weight, TPSA= Topological polar surface area, HBA= hydrogen bound acceptor, HBD= hydrogen bound donor.

The physicochemical characteristics of the three selected metabolites are conferred in Table 7. Giving to Table 7, the insolubility, lipophilicity, size, unsaturation, polarity, and flexibility of Vitexin, Beta-carotene, and Ferulic acid were analyzed. The results for Ferulic acid fell within acceptable limits, indicating that Ferulic acid possesses a favorable physicochemical profile, a crucial factor to monitor in pharmaceuticals and clinical studies. However, Vitexin and beta-carotene did not meet all the criteria.

**Table 8**. Predicted pharmacokinetics parameters of selected compounds by pkCSM server.

| Properties | Parameters | Vitexin | beta-carotene | Ferulic acid |
|---|---|---|---|---|
| **Absorption** | Water solubility | -2.845 | -7.39 | -2.817 |
| | GI Intestinal absorption (human) | 46.695 | 91.732 | 93.685 |
| | Log *K*p (skin permeation) cm/s | -2.735 | -2.741 | -2.72 |
| **Distribution** | BBB | -1.449 | 0.938 | -0.239 |
| | CNS permeation (Log PS) | -3.834 | -1.094 | -2.612 |
| | VD (human) | 1.071 | 0.266 | -1.367 |
| **Metabolism CYP2D6** | CYP1A2 inhibitor | ** | ** | ** |
| | CYP2C9 inhibitor | ** | ** | ** |
| | CYP2C19 inhibitor | ** | ** | ** |
| | CYP3A4 inhibitor | ** | ** | ** |
| | CYP2D6 inhibitor | ** | ** | ** |
| **Excretion** | Total Clearance (CL) (log mL/min/kg) | 0.444 | 1.061 | 0.623 |
| | Renal OCT2 substrate | No | No | No |

**= NO effect, OCT2= organic cation transporter 2, VD= Volume of distribution, BBB= Blood-brain barrier, VDss=steady state volume of distribution, GI= Gastrointestinal, CL= Range: 5mL/min/kg< Cl < 15mL/min/kg: >15 mL/min/kg: high; moderate; <5 mL/min/kg: low.
logBB>0.3 is considered too readily cross the BBB.
logBB <-1 are unsuccessfully delivered to the brain.
VDss is deemed low if less 0.71 L/kg (log VDss <-0.15) and exalted if over 2.81 L/kg (log VDss> 0.45).
CNS permeability with logPS> -2 is considered to penetrate the CNS, while logPS<-3 is reflected as incapable to enter CNS.

**Table 8** presents the pharmacokinetic parameters including gastrointestinal absorption (HIA), BBB, CNS permeation (Log PS), volume of distribution (VD) in humans, and inhibition of CYP isoforms. The molecules were assessed for their properties as inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP3A4, CYP2D6, and their Log Kp (skin permeation). Absorption is a crucial parameter that is evaluated for every biomolecule before it is formulated into a drug for pharmaceutical or clinical trials [50, 51]. BBB penetration is crucial for compounds that target the CNS as they need to cross the BBB. Inactive compounds, on the other hand, should not cross the BBB to prevent adverse effects on the CNS [50].
As shown in Table 8, ferulic acid exhibited a HIA with BBB permeability, representing a low likelihood of harmful CNS impacts. Other compounds showed low HIA with no BBB permeability.
Beta-carotene exhibited a lower skin permeation Log Kp relative to other compounds. A more negative Kp value signifies decrease permeability of the biomolecule over the skin barriers. The five primary CYP isoforms show a vital role in the elimination of pharmaceuticals and are involved in the metabolism of approximately 75% of the medications on the market. Restraining any of these isoforms can lead to substantial drug-drug interactions [29, 48]. Fifty to ninety percent of biomolecules are substrates of the five main isoforms: CYP3A4, CYP2D6, CYP2C19, CYP2C9, and CYP1A2. Inhibition of these isoenzymes is a general reason of pharmacokinetics-connected drug-drug interfaces, leading to toxic ADME due to the accumulation of drugs/metabolites.
All mentioned metabolites did not inhibit any CYP isoform and were rapidly metabolized. Furthermore, the clearance value of the three selected compounds was insufficient, indicating low clearance. The use of OCT2 inhibitors and substrates together may result in unfavorable interactions, and it has been assumed that the compounds would not act as substrates for OCT2. The boiled egg plot for three compounds was accomplished to further confirm the GI absorption and BBB permeating actions of the three hit biomolecules **Figure 12.**

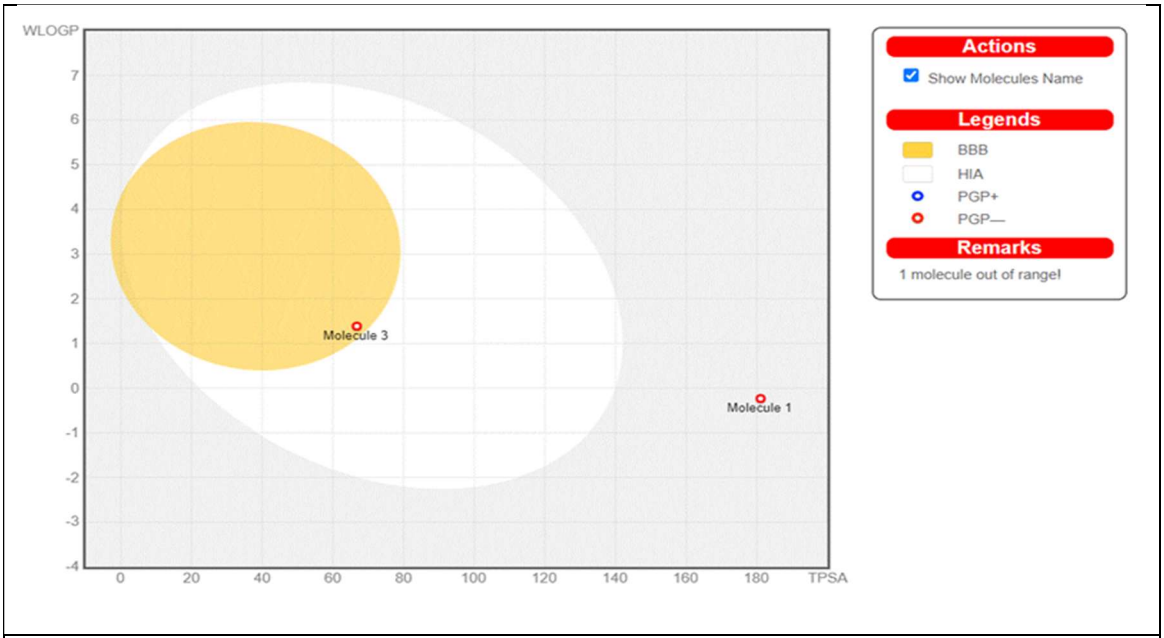



**Figure 12.** The BOILED-egg plot for brain permeation prediction and gastrointestinal tract absorption of the three chosen biomolecules

The white region represents a high probability of passive absorption by the gastrointestinal tract, while the yellow region (yolk) indicates a high probability of brain penetration. The yolk and white areas are not mutually exclusive. Additionally, points are colored blue if predicted to be actively effluxed by P-gp (PGP+) and red if predicted to be a non-substrate of P-gp (PGP−).
Figure 12 illustrates the relationship between WLOGP and TPSA, which can be used to predict the gastrointestinal absorption and brain penetration of the selected molecules. In this case, the vitexin compound (referred to as molecule 1) and ferulic acid (referred to as molecule 3) fall within the range, while beta-carotene is outside the range.

Molecule 1 demonstrates low human gastrointestinal absorption (GI), whereas molecule 3 and beta-carotene show high human gastrointestinal absorption (GI), indicating passive absorption by the gastrointestinal tract. Neither molecule 1 nor beta-carotene exhibit a BBB feature for CNS penetration, except for molecule 3. Molecule 1 and molecule 3 have been not evaluated as P-glycoprotein substrates (PGP-) for the central nervous system. Based on the above discussions, it is evident that beta-carotene is highly pharmacologically active, with good absorption and oral bioavailability.

### 3.7. Prediction of the Bioactivity Score

The bioactivity score is applied to estimate the drug-like properties of ligands such as KI, NRL, GPCR, ICM, EI, and PI. The Molinspiration Cheminformatics software was used to determine the bioactivity scores of vitexin, beta-carotene, and ferulic acid. These scores are crucial for evaluating

the drug-like characteristics of ligands. A score above 0.00 denotes high activity, while scores between -0.5 and -0.00 indicate moderate activity. Scores below -0.5 suggest inactivity [52]. The secondary metabolites under study exhibited high to moderate activity, except for Ferulic acid, which showed inactivity in KI and PI with scores of -0.72 and -0.81, respectively. Vitexin had the highest score across all parameters, except for ICM, showing moderate activity with a score of -0.14.

Beta-carotene exhibited the highest score of 0.40 for NRL and 0.17 for EI, while the other four parameters showed moderate action. In comparison, Dovitinib demonstrated good scores for all features, whereas Gefitinib displayed inactivity in some actions, as shown in Figure 13. A great bioactivity score suggests that these bio actives have the potential to be potent therapeutic agents, with greater scores indicating higher activity.

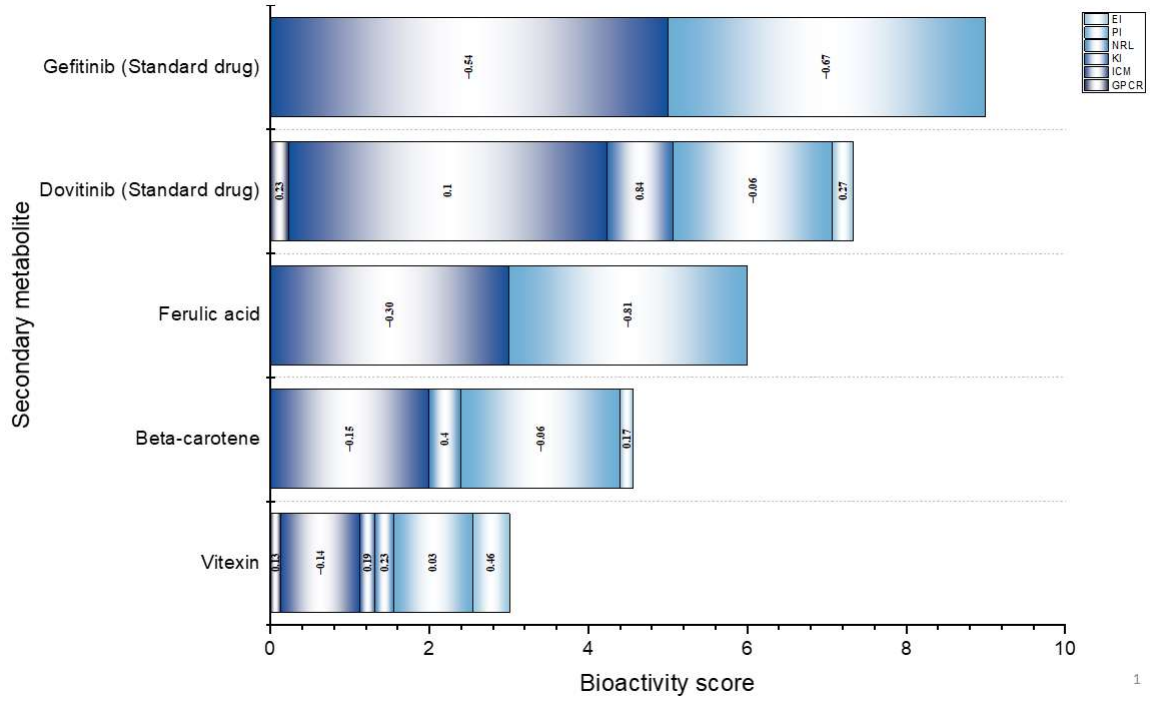
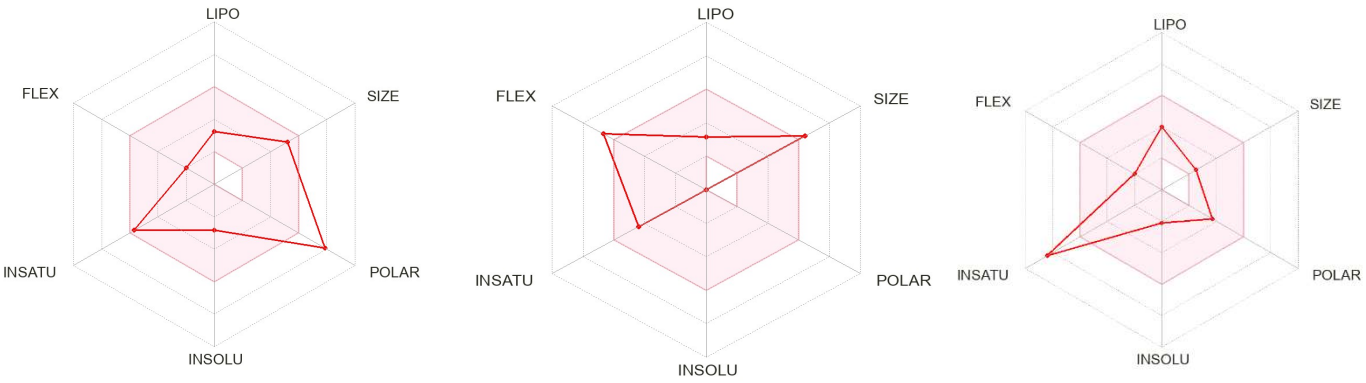



**Figure 13.** Bioactivity score of secondary metabolites Vitexin, Beta-carotene, and Ferulic acid according to the Molinspiration software.

### 3.8. Bioavailability Radar

The bioavailability radar quickly evaluates the compound's suitability as a drug. The pink area in Figures 14 illustrates the ideal range for each parameter. When evaluating a compound's parameters, the radar plot must align within the pink area to be classified as drug-like. Thus, the radar plot predicts whether the ligands are likely to be orally bi-available or not. Polarity (polar) and Flexibility (FLEX) are two crucial properties that play a significant role in determining the bioavailability of compounds. FLEX is established by rotatable bonds; molecules with more than

10 rotatable bonds are predicted to have low oral bioavailability. The topological polar surface area (TPSA) determines polarity [53], indicating that compounds with a TPSA exceeding 20 Å² but less than 130 Å² exhibit high oral bioavailability [38, 49] . In comparison to other compounds, Ferulic acid stands out as it meets the radar plot criteria, suggesting potential oral bioavailability. Vitexin, with a TPSA of 181.05, is also considered to exhibit high oral bioavailability, while beta-carotene has a TPSA of 0.00.




**(a)** **(b)** **(c)**

**Figure 14.** Radar plots of different ligands: (a) vitexin, which is predicted to be not orally bioavailable due to being too polar; (b) beta-carotene, which is predicted to be not orally bioavailable due to being too flexible; and (c) ferulic acid, which is predicted to be orally bioavailable.
The pink area indicates the ideal range for each property: polarity (TPSA: 20-130 Å2), lipophilicity (XLOGP3: -0.7 to +5.0), solubility (log S ≤ 6), size (MW: 150-500 g/mol), saturation (fraction of sp3 hybridization carbons ≥ 0.25), and flexibility (≤ 10 rotatable bonds).

### *3.9. Cardiac toxicity analysis*

In order to thoroughly assess the toxicity of the discovered metabolites, it was essential to examine their ability to inhibit the hERG channel, which is recognized for its potential to induce cardiac toxicity. A study on cardiac toxicity was handled utilizing the cardioToxCSM and pred-hERG 5.0 Webserver. This exploration sought to estimate the metabolites' capacity to prevent the hERG cardiac potassium channel and potentially cause harmful cardiovascular consequences. The FDA delegates that all biomolecules undergo hERG safety testing before being considered as a therapeutic candidate. Blockage of hERG has been connected to fatal cardiac arrhythmias [54]. According to the results from pred-hERG 5.0, none of the metabolites displayed any indications of cardiac toxicity, as shown in **Table 9.**

**Table 9.** Assessment of cardiac toxicity in the detected biometabolites employing the pred-hERG 5.0 Webserver.

| Metabolite Name | Activity on hERG channel | Confiability % |
|---|---|---|
| Vitexin | Non-blocker | 91.81 |
| Beta-carotene | Non-blocker | 99.38 |
| Ferulic acid | Non-blocker | 97.58 |

**Table 10** identifies six of the most frequent clinical cardiac toxicity outcomes for all three selected compounds. Ferulic acid showed positive results on all six clinical cardiac toxicity outcomes. In contrast, the other compounds exhibited some level of toxicity. Vitexin displayed toxic effects on hERG toxicity and myocardial infarction. On the other hand, beta-carotene was associated with toxicity in hERG toxicity and hypertension.

**Table 10.** Assessment of cardiac toxicity in the detected biometabolites employing the cardioToxCSM Webserver.

| Metabolite | Cardiac Toxicity signs | | | | | |
|---|---|---|---|---|---|---|
| | Arrhythmia | Cardiac failure | Heart block | hERG toxicity | Hypertension | Myocardial infarction |
| **Vitexin** | S | S | S | T | S | T |
| **beta-carotene** | S | S | S | T | T | S |
| **Ferulic acid** | S | S | S | S | S | S |

S=Safe; T= Toxic

### *3.10. Prediction of Cell Line Cytotoxicity*

In the following text, the in-silico prediction of cytotoxicity for cancer cell lines is detailed, showcasing the cytotoxic capability of trained hits reported in expressions of their Pa and Pi values against cancer cell lines. The results indicate that shortlisted secondary metabolites demonstrated action opposed to numerous cancer cell lines at Pa > 0.5, as this threshold was deemed to show significant differences.

The observed results suggest potential cytotoxic activities for both compounds. Notably, beta-carotene showed the highest Pa score of 0.889 against Carcinoma. A higher Pa score indicates a

higher prospect of a molecule being cytotoxic, while the Pi score signifies the inactivation probability. Importantly, the Pa values of all compounds against the mentioned cell lines are notably greater than the Pi values. Hence, these results suggest potential cytotoxic actions for these biomolecules, with the Pa and Pi values of the qualified molecules clarified in **Table 11.**
Furthermore, both beta-carotene and Ferulic acid demonstrated the best significant scores at a better cut-off value of Pa > 0.5, showing the highest scores for the Carcinoma and Leukemia cell lines (PC-3, K562) respectively. Conversely, Vitexin presented the greatest scores for the Leukemia cell line (HL-60). Additionally, these predictions encompass cytotoxicity against several cell lines, which is supported by relevant publications.

**Table 11.** In silico prediction of cell line cytotoxicity for secondary metabolite by CLC-pred 2.0.

| **Metabolite Name** | **Cell line** | **Cell line full name** | **Tissue** | **Tumor type** | **Pa** | **Pi** |
|---|---|---|---|---|---|---|
| **Vitexin** | HL-60 | Promyeloblast leukemia | Hematopoietic and lymphoid tissue | Leukemia | 0.696 | 0.009 |
| **Beta-carotene** | CWR22R | Prostate carcinoma epithelial cell line | Prostate | Carcinoma | 0.720 | 0.003 |
| | PC-3 | Prostate carcinoma | Prostate | Carcinoma | 0.889 | 0.004 |
| | MDA-MB-231 | Breast adenocarcinoma | Breast | Adenocarcinoma | 0.540 | 0.022 |
| | LNCaP | Prostate carcinoma | Prostate | Carcinoma | 0.660 | 0.003 |
| | U-266 | Plasma cell myeloma | Blood | Myeloma | 0.526 | 0.006 |
| | T47D | Breast carcinoma | Breast | Carcinoma | 0.514 | 0.017 |
| **Ferulic acid** | K562 | Erythroleukemia | Haematopoietic and lymphoid tissue | Leukemia | 0.542 | 0.020 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | IGROV-1 | Ovarian adenocarcinoma | Ovarium | Adenocarcinoma | 0.529 | 0.016 |

Pa > 0.3, Pi: probability to be inactive; Pa: probability to be active.

### *3.11. Toxicity profile Evaluation*

A range of toxicities, encompassing effects on human health and the environment, were evaluated for each molecule (see **Table 12**). The mutagenic possibility of a compound can be uncovered through the Ames test. Vitexin and Ferulic acid were both categorized as non-Ames hazardous, indicating uncertainty regarding their carcinogenic properties, except for beta-carotene based on the findings.

The maximum tolerated dose (MTD) in humans supplies as a display of a chemical's toxicity level. Compared to the other three compounds, the MTD for Ferulic acid was significantly higher. Inhibition of the potassium channels encoded by the hERG gene could potentially lead to catastrophic ventricular arrhythmia. As shown in the table below, all three metabolites can hinder hERG I, but not hERG II for beta-carotene. Additionally, none of the tested chemicals exhibited hepatotoxic effects, which could result in drug-induced liver damage. Dermally administered products may potentially cause skin hypersensitivity, but none of the substances investigated demonstrated the ability to induce skin sensitization in humans. Moreover, both Vitexin and beta-carotene were classified as class 4 for the prediction of lethal dose (LD50), indicating that they are "harmful if swallowed" ($300 < LD50 \leq 2000$), while Ferulic acid was categorized as class 5, indicating that it "may be harmful if swallowed" ($2000 < LD50 \leq 5000$) (refer to **Table 13).**

**Table 12.** Predicted toxicity profile of selected compounds by GUSAR and pkCSM server.

| **Parameters** | **Vitexin** | **beta-carotene** | **Ferulic acid** |
|---|---|---|---|
| **AMES toxicity** | No | Yes | No |
| **Max. tolerated dose (human)** **(log mg/kg/day)** | 0.577 | -0.379 | 1.082 |
| **hERG I Inhibitor** | No | No | No |
| **hERG II Inhibitor** | No | Yes | No |
| **Oral Toxicity (LD50) (mg/kg)** | 5531,000 | 7404,000 | 2754,000 |
| **Oral Toxicity classification** | VI | VI | V |

| | | | |
|---|---|---|---|
| **Hepatotoxicity** | No | No | No |
| **Skin Sensitisation** | No | No | No |
| ***T.Pyriformis* toxicity** | 0.285 | 0.326 | 0.271 |
| **Minnow toxicity** | 4.897 | -0.028 | 1.825 |

Class I: fatal if swallowed (LD50 ≤ 5); class V: may be harmful if swallowed (2000 < LD50 ≤ 5000); class III: toxic if swallowed (50 < LD50 ≤ 300); class II: fatal if swallowed (5 < LD50 ≤ 50); class IV: harmful if swallowed (300 < LD50 ≤ 2000); and class VI: non-toxic (LD50 > 5000).

### 3.12. Biological activity analysis

The web server can predict biological activity by analyzing the ligand's structure. In this study, the prediction was applied to three selected compounds. All three compounds showed the same biological action, with the possibility of the ligands acting as antineoplastic drugs, which have tumor-restrictive properties, ranging from 0.573 to 0.430 when Pa > Pi (**Table 13**).

**Table 13:** Biological action prediction of the chosen three ligands.

| Metabolite Name | Pa | Pi | Biological activity prediction |
|---|---|---|---|
| **Vitexin** | 0.430 | 0.027 | Antineoplastic (breast cancer) |
| **Beta-carotene** | 0.573 | 0.013 | Antineoplastic (breast cancer) |
| **Ferulic acid** | 0.467 | 0.023 | Antineoplastic (breast cancer) |

Pa = probability of being active; Pi = probability of being inactive.

When Pa is greater than 0.7, the constituent is greatly likely to demonstrate activity in the test, but it also has a high chance of being an analog of a known pharmaceutical mediator. If Pa falls between 0.5 and 0.7, the ingredient is still likely to exhibit activity, although the probability is lower, and it differs from known pharmaceutical agents. If Pa is less than 0.5, the substance is unlikely to exhibit activity in the experiment. However, if the activity is confirmed, the substance could potentially be a new chemical entity. The Pa values indicated a higher likelihood of activity when Pa was greater than 0.5, whereas the probable inactivity (Pi) scores were almost 0, indicating a high expectation for the compound to exhibit these activities. **Table 14** displays the most favorable outcomes, categorizing Antineoplastic as the best predicted activity for three selected

compounds [55]. Beta-carotene showed the most significant predicted activity (Pa) for biological activity as antineoplastic with a Pa between 0.5 and 0.7. On the other hand, Ferulic acid and Vitexin, with Pa values lower than 0.5, are unlikely to demonstrate activity in the experiment.

### *3.13. Organ and Endpoint Toxicity Assessment*

Various toxicity parameters of the identified metabolites were extensively evaluated to confirm their safety and appropriateness for anti-cancer use. A detailed toxicity assessment was carried out, and the findings are outlined in Table 14. Among the metabolites analyzed, Vitexin and beta-carotene exhibited significant activity, with a predicted mutagenicity probability of 52% and 70%, respectively. Additionally, ferulic acid was found to possess an immunotoxicity of 91%. Furthermore, none of the three selected metabolites were found to be hepatotoxic, carcinogenic, or cytotoxic. Accordingly, these results present valuable insight into the safety profile of the classified metabolites that may be useful in the development of anticancer compounds in the future.

**Table 14.** Comprehensive Toxicity Assessment of the Identified Metabolites using Protox II Webserver.

| Metabolite Name | Classification | | | | |
|---|---|---|---|---|---|
| | **Organ Toxicity (%Probability)** | **Toxicity Endpoint (% Probability)** | | | |
| | **Hepatotoxicity** | **Carcinogenicity** | **Immunotoxicity** | **Mutagenicity** | **Cytotoxicity** |
| **Vitexin** | 0.81* | 0.72* | 0.82* | 0.52** | 0.87* |
| **Beta-carotene** | 0.85* | 0.86* | 0.88* | 0.71** | 0.81* |
| **Ferulic acid** | 0.51* | 0.61* | 0.91** | 0.96* | 0.88* |

*=Inactive, ** = active

In recent years, there has been a extending interest in investigating natural compounds for their potential bioactive properties in combating various diseases, particularly cancer. Aspergillus sp has emerged as a significant source of biologically active metabolites with diverse beneficial effects, containing anti-inflammatory, antiviral, antimicrobial, and anticancer actions [56]. Several studies have emphasized the valuable role of *Aspergillus sp* as a source of novel bioactive natural products with antiproliferative activity against several cancer cells [57].

Notably, in our in-silico studies, we identified three secondary metabolites - vitexin, beta-carotene, and ferulic acid - derived from Aspergillus sp, which exhibited promising anticancer properties against human cancer cells [19]. Our in-silico analysis revealed that these secondary metabolites demonstrated notable binding energies against key cancer-related proteins, including BRCA1, BRCA2, and TP53. Specifically, vitexin displayed the highest binding energy with all three target proteins, indicating its strong potential as an anticancer agent. Prior to clinical trials or production, it is essential to assess the toxicological profile of potential medicines. According to RO5 analysis, ferulic acid met all five criteria, indicating its favorable drug-like properties. While beta-carotene did not fully satisfy the criteria, vitexin showed a deviation in at most one criterion, which is still considered acceptable.

Based on bioactivity scores, vitexin exhibited high activity for various properties, indicating its potential as an effective therapeutic agent. Moreover, our analysis suggested that ferulic acid is likely orally bioavailable, further emphasizing its promise as an anticancer compound. Our findings indicated that vitexin, beta-carotene, and ferulic acid demonstrated significant activity in terms of cell line cytotoxicity, with vitexin showing high scores for the Leukemia cell line (HL-60). Importantly, none of the three selected metabolites exhibited signs of cardiac toxicity, Hepatotoxicity, Carcinogenicity, or Cytotoxicity, providing valuable insights into their safety profile. In line with our findings, a study by [58] reported the anti-apoptotic effect of vitexin in various cell lines, including breast, ovarian, and prostate, further supporting the potential of vitexin as an anti-cancer agent.

Additionally, research by [59] highlighted vitexin's induction of apoptosis in MCF-7 Breast Cancer Cells through the regulation of specific miRNAs ex-pression, corroborating our in-silico results and previous research on vitexin's potential for anticancer therapy. In conclusion, our in-silico analysis, coupled with existing research, underscores the promising future of vitexin as a potential anti-cancer drug, further supporting its development as a valuable addition to anticancer therapy [60,61].

## 5. Conclusion

Fungal-derived natural products have a wide range of biological activities, making them a valuable alternative to synthetic compounds. In a previous study, highly effective compounds with potential anticancer properties were identified in the fungus A. flavus. In this present study, we selected three potent compounds and evaluated their effects on three tumor suppressor proteins in order to find a promising drug candidate for breast cancer. We employed computational techniques such as molecular docking, ADMET analysis, pharmacological profiling, and cardiac toxicity assessments.

After performing multiple docking experiments, we can conclude that vitexin has potent effects on cancer proteins (BRCA1, BRCA2, and TP53) compared to other compounds. Both vitexin and ferulic acid successfully docked and confirmed the highest binding energy with the BRCA2 protein. In addition to its strong binding energies towards the targeted proteins, vitexin also

demonstrated safety in cardiac and hepatotoxicity assessments, as well as suitable physicochemical and pharmacokinetic properties.

Overall, our findings suggest that vitexin, isolated from *A. flavus,* holds promise as a candidate for the development of anticancer drugs, and further experimental exploration is warranted. Furthermore, the comprehensive pharmacological profile provided, which includes PASS predictions, bioactivity scores, ADMET assessments, and molecular docking results, lays the groundwork for investigating other bioactive compounds in the future, targeting various types of cancers.

**Author Contributions:** "Conceptualization, B.S. and S.A.; methodology, S.A.; software, S.A.; validation, B.S. and S.A.; formal analysis, S.A.; investigation, B.S.; resources, B.S.; data curation, S.A.; writing—original draft preparation, S.A.; writing—review and editing, B.S.; visualization, B.S.; supervision, B.S.; project administration, B.S.

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