Molecular Docking and ADMET Study of Phytochemicals as Anticancer Agent towards Alpha 1-Antichymotrypsin Variant DBS-II and Oxidised Quinone Reductase-2

ABSTRACT

Objective: The main goals of this study were to do molecular docking and absorption, distribution, metabolism, excretion, and toxicity (ADMET) tests on vanillic acid, ferulic acid, chlorogenic acid, and catechin hydrate. The study aimed to determine the receptor affinity of these drugs using molecular docking simulations, with a focus on the potential anticancer impact of targeting the Alpha 1-Antichymotrypsin Variant DBS-II and Oxidised Quinone Reductase-2.

Materials and Methods: Doxorubicin was used as a reference standard for the molecular docking investigations of the compounds, which were performed using Protein Data Bank (PDB) ID: 4ZVM and PDB ID 5OM7. Additionally, we performed ADMET investigations of these drugs using SwissADME and ProTox-II software programmes.

Results: Chlorogenic acid exhibited a higher score than the other three compounds with regard to anticancer activity, with scores of -7.869 Kcal/mole for oxidised quinone reductase-2 in complex and -5.941 Kcal/mole for Alpha1-Antichymotrypsin Variant DBS-II, respectively, after the reference drug. ADMET analysis demonstrated the suitability of these compounds for use in pharmaceutical applications.

Conclusion: Studies show that all four drugs bind to Oxidised Quinone Reductase-2 (OQR-2) and Alpha 1-Antichymotrypsin Variant DBS-II, which could mean that they can fight cancer. In addition, the toxicity evaluation and ADME analysis reveal how suitable these substances are for use in pharmaceuticals.

Keywords: Anticancer Activity; Alpha 1-Antichymotrypsin Variant DBS-II; Oxidised Quinone Reductase-2; Molecular Docking; ADMET Properties

1. INTRODUCTION

Genetic mutations that disrupt normal control over cell division are the root cause of cancer, a complex disease with uncontrollably fast development and the ability to penetrate and spread. Cancer is characterised by prolonged proliferative signalling, evading immune eradication and growth inhibitors, allowing for replicative immortality, inducing angiogenesis, starting invasion and metastasis, affecting energy metabolism, and avoiding cell death. There are several possible reasons for such alterations, including personal lifestyle choices, long-term inflammation, and environmental pollutants. Furthermore, a multitude of cancer types may be classified based on the original cell type that gave rise to the illness. For example, carcinoma targets epithelial cells, leukaemia affects blood cells, sarcoma affects connective tissues, and lymphoma affects the lymphatic system [1,2].

Natural substances that can prevent cancer include vanillic acid, ferulic acid, chlorogenic acid, and catechin hydrate [3–9]. Many mechanisms have been shown to support the anticancer effects of chlorogenic acid, which is abundant in coffee but also present in fruits and vegetables. These mechanisms include the induction of controlled cell death in cancer cells, the inhibition of their migration and proliferation, the suppression of angiogenesis, and the modification of the cell cycle [2,3]. Ferulic acid is a diverse phytochemical that is present in many different plants than coffee. Because of its focus on certain signalling pathways that regulate the growth and survival of malignant cells, it may have anticancer properties. It may also cause cell cycle arrest, lessen the motility and viability of cancer cells, and increase the efficacy of cancer therapies [4,5]. Vanillic acid, the main component of vanilla, gives it its unique scent and a surprising number of possible health benefits. According to studies, it may possess anticancer

qualities by inducing apoptosis, preventing cancer cells from proliferating and dividing, and altering genes linked to the development and spread of cancer [7,8]. Moreover, a kind of flavanol called catechin hydrate, which is present in green tea, grapes, and other plants, has the potential to prevent the growth and multiplication of cancer cells, cause apoptosis, lessen angiogenesis, and modify the immune system, among other possible anticancer effects [9]. To fully understand the potential of these chemical molecules for future cancer prevention and therapeutic programmes, more research is required.

Oxidised quinone reducase-2 (NQO2), also known as NRH: quinone oxidoreductase 2, is an enzyme found in the cytosol, or fluid-filled part, of cells. It is important to cellular detoxification because it catalyses the reduction of quinones, which are a class of reactive chemicals synthesised during normal metabolism or exposure to environmental contaminants. These quinones have the potential to harm biological components and induce oxidative stress if they are not managed. NQO2 works as a cellular defence mechanism, efficiently counteracting the adverse effects of the potentially harmful quinones by converting them into less reactive hydroquinones [10]. Apparently, NQO2 cannot be just used for detoxification; studies suggest that it could also affect cellular signalling pathways and the course of cancer [11]. NQO2 activity can have an impact on apoptosis, a regulated cell death process that is necessary for getting rid of damaged or undesirable cells. Some studies suggest that NQO2 may increase the chance of survivability for healthy cells by prolonging the apoptotic process [12]. NQO2 may, however, have the opposite impact on cancer cells, accelerating the cell's death through inhibition [13]. Cells have a balance between the production and elimination of reactive oxygen species (ROS), and NQO2 helps to keep this balance. High levels of ROS are linked to the development of cancer. The potential effect of NQO2 on cancer risk is further boosted by its ability to reduce quinones [14,15]. In addition to assisting in detoxification, NQO2 protects cells against quinones' adverse impacts on DNA. However, other research

indicates that NQO2 activity may inadvertently produce DNA-damaging intermediates in some situations, which might lead to the formation of cancer [13].

Human blood plasma contains the protein known as alpha-1 antichymotrypsin (A1ACT); however, due to possible drug interactions, a particular form of the protein known as alpha-1 antichymotrypsin variant DBS-II (A1ACT DBS-II) is gaining attention. The main action of A1ACT is to block chymotrypsin, a digestive protease that breaks down peptides in the intestines. A1ACT prevents excessive tissue damage from unregulated protein breakdown by controlling chymotrypsin activity [16,17]. A1ACT DBS-II is a protein variant that differs from the original A1ACT in terms of its three-dimensional structure. This structural variation arises from a specific replacement of amino acids throughout the protein chain. With variations in their chemical structures, A1ACT DBS-II may still be competent to interact with a wide range of pharmacological drugs, including those that are in the anthracycline class. One of the most common chemotherapeutic agents in this group is doxorubicin. Studies using protein crystallographic techniques have demonstrated that A1ACT DBS-II may bind to doxorubicin and form complexes that may affect the drug's systemic distribution as well as its effectiveness [18,19].

Once associated with a chemotherapeutic agent, A1ACT DBS-II could function as a vehicle for transport. It might deliver the drug to specific physiological components that are suitable for targeting cancer cells [19]. A1ACT DBS-II can increase the drug's intensity at the tumour site, which may lead to more efficient cancer cell killing. This allows for the systematic delivery of the chemotherapeutic agent to cancer cells so as to improve drug delivery to cancer cells.

The objective of this study was to ascertain the ability of vanillic acid, ferulic acid, chlorogenic acid, and catechin hydrate to associate with Oxidised Quinone Reductase-2 (OQR-2) and the Alpha 1-Antichymotrypsin Variant DBS-II and their possible contribution to the anticancer

activity. A computational approach based on molecular docking was used to investigate these naturally occurring chemicals and the previously mentioned targets in order to search for possible binding interactions. By comparison with reference standards, the relative strength and comparative efficiency of these drugs were determined.

2. MATERIALS AND METHODS

To evaluate ligand binding in silico docking studies, Schrödinger's Glide v9.1 tool was utilised. Following that, the MMGBSA/SA method was used to calculate the binding free energies. The potential toxicity and ADME characteristics of the ligands from the MM/GBSA study were evaluated using software programmes such as SwissADME and ProTox-II. Every computational approach employed in this study will be covered in detail in separate sections.

2.1 Preparation of proteins:

In this work, ligand and reference molecules were chosen, and docking of molecules was carried out using publicly available protein structures from the Protein Data Bank (PDB ID: 4xum and 3vln) (http://www.resb.org). To prepare the protein for docking, bond ordering was established, co-factor formal charges were applied, and missing hydrogen atoms were added. Internal ligand preparation was carried out, and unwanted interconnections between ligand and protein atoms were removed. Eventually, a relaxed state for the protein complex that resembled its crystallographic structure was guaranteed by energy reduction utilising the OPLS 2005 forcefield. Molecules of water near 5 Å were also eliminated. Using the Protein Preparation Wizard's default parameters and a 1.0 scaling factor, a receptor grid was created with a 10 Å radius around the co-crystallized ligand to identify the binding site for the compounds under study.

2.2 Ligand Preparation:

In this research, the e-Molecule database was utilised as the primary resource for ligands and other compounds. It was transformed into a readable SDF (structural data file). The Schrödinger software suite's LigPrep module was then used to preprocess these compounds. The primary objectives of this preparation stage were to produce a minimum of five low-energy stereoisomers for each ligand and ensure that the compounds adhered to the correct structural frameworks. LigPrep selection was done using default settings to take physiological variability into consideration. After preparation, the resulting ligands were subjected to high-throughput virtual screening using the Schrödinger suite's GLIDE module. A comprehensive analysis was made possible by the virtual screening technique utilised in this work, which created appropriately viable interactions between the ligands and the target molecule. The e-Molecule database provided the primary source of ligands and supplementary compounds for this research. These chemicals were initially transformed into a readable structural data file (SDF) format to allow for additional processing and analysis. These compounds were then pre-processed using the LigPrep module of the Schrödinger software package. The primary objectives of this preparation stage were to produce at least five low-energy stereoisomers for each ligand and to verify that the compounds conformed to the proper structural frameworks. Physiological variability was taken into account when selecting LigPrep by using the default values. After preparation, the resulting ligands were subjected to high-throughput virtual screening using the Schrödinger suite's GLIDE module. This virtual screening approach allowed for thorough investigation and evaluation by simulating plausible interactions between the ligands and the target molecule.

2.3 Molecule docking:

The Glide application (version) of Maestro's receptor grid creation features was used to construct the receptor grid. After the ligands were ready, they underwent a three-step Glide docking process. "High Throughput Virtual Screening" (HTVS), the initial step, is a quick

screening technique that effectively selects viable ligand combinations from an extensive database. Ligands achieving high HTVS scores were then advanced to the more strenuous "Extra Precision" (XP) mode. A more precise, however intensively computational, evaluation of ligand-protein interactions is offered by XP mode. Furthermore, the docked conformations, which display the various ligand orientations inside the protein binding pocket, were assessed using the Glide score (dG score). Stronger interactions are indicated by higher scores. The estimated binding affinity between a ligand and a protein is indicated by this score.

2.4 MM/GBSA:

The approach of MM-GBSA was utilised to ascertain the respective binding energies of the chosen ligands. The XP docking findings, in the form of a pv.maegz file, served as the source of input for the MM-GBSA analysis. The protein's active site was arranged to accept changes up to five angstroms away from the ligand. This approach provides a comprehensive analysis of ligand-protein interactions, taking into consideration the flexibility of the active site and providing valuable insights into the energetics of ligand binding.

Lower (more negative) total binding free energies are predicted to interact with the target protein more frequently, making them intriguing candidates for more research and examination.

2.5 ADMET Prediction:

ProTox-II, a toxicity prediction software programme, was utilised in the study to estimate the likely toxicity of molecules. Using SwissADME, the absorption, distribution, metabolism, and excretion (ADME) properties of the most promising ligands found by the MM-GBSA results were carefully assessed. A thorough analysis was then presented, along with a variety of ADMET property predictions that are essential to understanding the pharmacokinetic characteristics of the drugs and their therapeutic applications.

3. RESULTS

3.1 Molecular docking studies:

Computational analysis was used using substances that showed well-predicted IC50 values in order to confirm the theory. When predicting the interactions between molecules, such as new tiny hits or ligands, and a target macromolecule, usually a protein, molecular docking plays a critical role. Part of this process involves predicting the affinities and mechanisms of ligand-protein binding. Oxidised quinone reductase 2 in complex (PDB ID: 4ZVM) and drug-binding serpin Alpha1-antichymotrypsin variant DBS-II (PDB ID: 5OM7) were produced in conjunction with doxorubicin for the production of proteins and the formation of receptor grids relevant to anticancer activity. The Glide v9.1 receptor grid construction panel was used to generate a grid surrounding the active region of the protein after the protein structure was processed using the protein preparation wizard panel. Consequently, it was ensured that the protein structure would be ready for further docking and computer analysis.

3.2 Ligand Preparation:

To optimise the conformations of the compounds, geometric minimization using the OPLS 5 force field was performed after the compounds were first shown using the 2D sketcher tool. LigPrep was used to guarantee that compound structures were refined for further study. This important step improves computational studies' accuracy and produces a more trustworthy representation of the molecular properties and interactions inside the molecules.

3.3 Ligand Docking with Glide:

When the Glide programme was launched, one of two docking methods, Extra Precision (XP) or Standard Precision (SP), was selected. XP offers more precision despite requiring more computing power. Setting parameters such as ligand flexibility, position sampling, scoring functions, and docking parameters were done. The molecular docking data, which show the ligands' binding affinities, are shown in Tables 1 and 2.

Table 1 : All molecules with their Dock Score and dG Binding (MM/GBSA) for PDB ID: 4ZVM

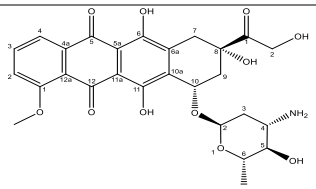
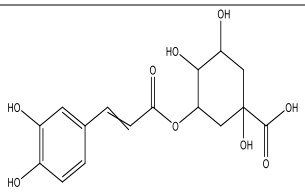
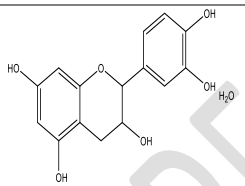
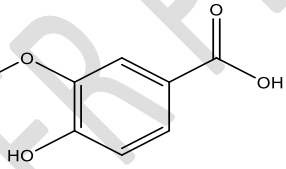
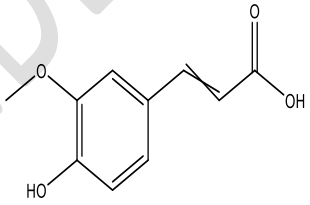
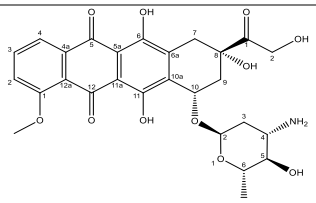
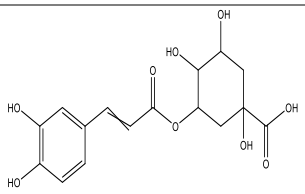
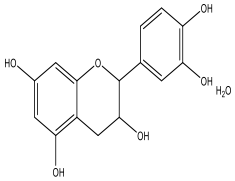
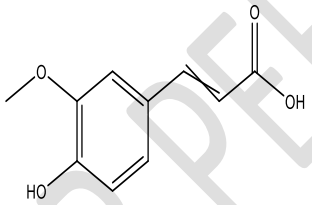
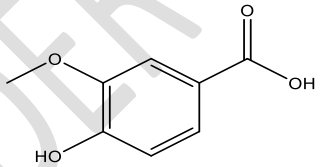
Compound name	2D structure	Dock Score Kcal/mole	dG Binding Kcal/mole
Reference Doxorubicin		-8.360	-67.26
Chlorogenic acid		-7.869	-48.53
Catechin hydrate		-6.544	-46.63
Vanillic acid		-4.688	-27.87
Ferulic acid		-3.777	-31.43

Table 2 : All molecules with their Dock Score and dG Binding (MM/GBSA) for PDB ID: 5OM7

Compound name	2D structure	Dock Score Kcal/mole	dG Binding Kcal/mole
Reference Doxorubicin		-7.702	-66.02
Chlorogenic acid		-5.941	-30.73
Catechin hydrate		-4.530	-37.37
Ferulic acid		-2.438	-8.12
Vanillic acid		-2.200	-6.82

3.4 MM-GBSA:

The technique of Prime/MM-GBSA was utilised to evaluate the relative binding energy of certain ligands. The protein's active site was configured to accommodate variations up to 5 Å from the ligand using the pv.maegz file from XP docking as the input for the MM-GBSA study. This method takes into consideration the flexibility of the active site while offering helpful

information on the energetics of ligand binding and enabling the in-depth examination of ligand-protein interactions. It is believed that molecules with lower (more negative) total binding free energies would be the best candidates for further investigation since they are more likely to interact meaningfully with the target protein. Tables 3 and 4 display the binding interactions of all compounds with the receptors for anticancer activity, Oxidised Quinone Reductase-2 and Alpha 1-Antichymotrypsin Variant DBS-II. Tables 5 and 6 provide interaction pictures of all compounds with Oxidised Quinone Reductase-2 and Alpha 1-Antichymotrypsin Variant DBS-II.

Table 3 : Binding interactions of all molecules with the receptors for Oxidized quinone reductase 2 in complex

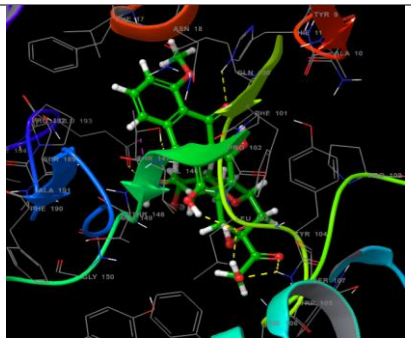
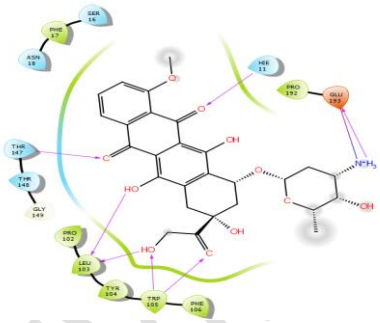
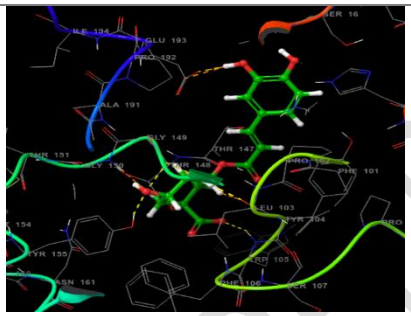
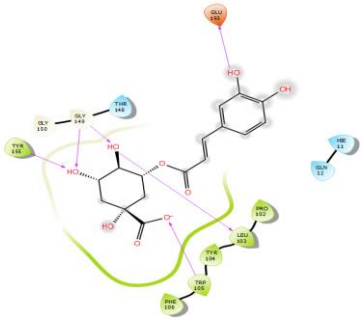
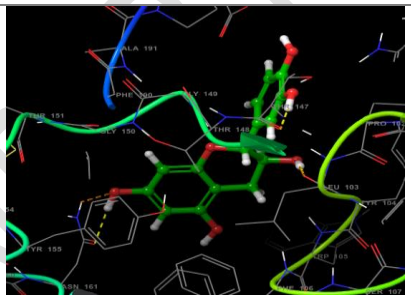
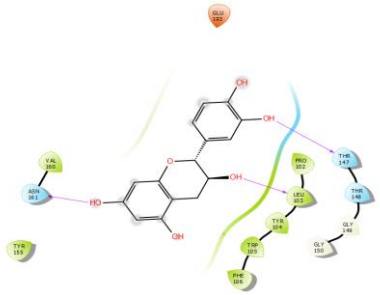
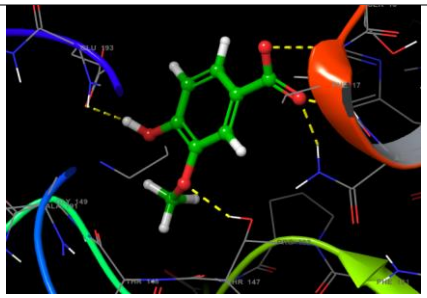
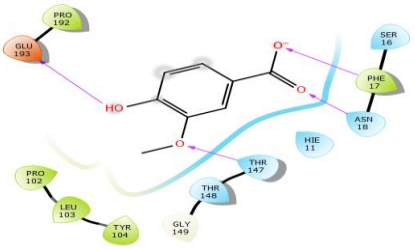
Compound Name	**2D Interactions**	**Distance (A^0)**
Reference Doxorubicin	H-Bond: GLU193,HIE11,TRP105,TRP105, LEU103,LEU103,THR147 Salt-Bridge:GLU193	1.90,2.40,2.19,1.96, 2.12,2.39,2.02
Chlorogenic acid	H-bond:TYR155,GLY149,GLY149,LEU103, GLU193,TRP105	1.91,2.68,1.97,1.93, 1.59,2.01

Catechin hydrate	H-bond:ASN161,THR147,LEU103	2.07,1.96,1.65
Vanillic acid	H-bond:ASN18,PHE17,THR147,GLU193	2.14,2.03,2.14,2.14
Ferulic acid	H-bond: TYR155,GLY150	1.83,2.09

Table 4 : Binding interactions of all molecules with the receptors for Alpha 1-Antichymotrypsin Variant DBS-II

Compound Name	**2D Interactions**	**Distance (A^0)**
Reference Doxorubicin	H-Bond:ASN244,PHE277,PHE277,GLU278 Pi-Pi Stacking:PHE277	2.18,2.05,2.03,1.85 3.93
Chlorogenic acid	H-bond:SER250,SER250,PHE277	1.84,1.98,1.96
Catechin hydrate	H-bond:SER250,SER250,GLN270,GLN270	2.51,2.37,2.06,2.23
Ferulic acid	H-bond:ASN244,PHE277	1.63,1.88
Vanillic acid	H-bond: ASN244,LEU280	2.73,1.87

Table 5 : Interaction images of all molecules with Oxidized quinone reductase 2 in complex with doxorubicin (PDB ID: 4ZVM)

Compound Name	3D Image	2D Image
Reference Doxorubicin		
Chlorogenic acid		
Catechin hydrate		
Vanillic acid		

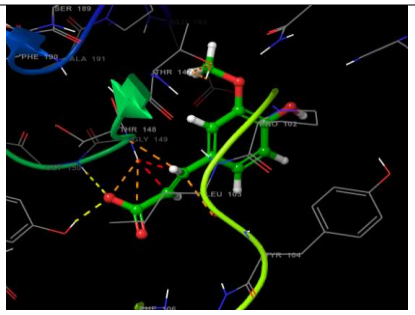
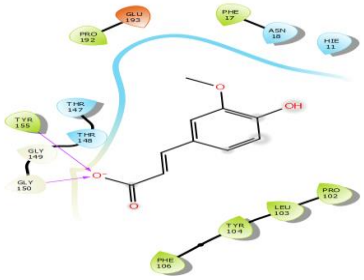
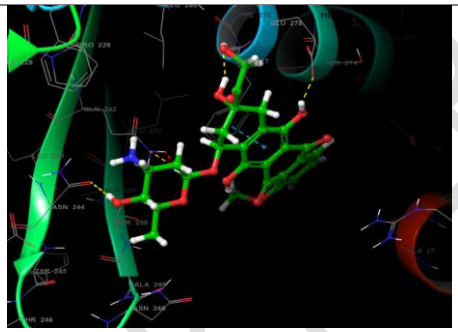
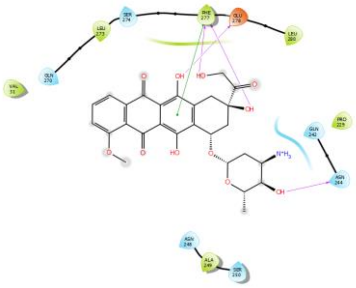
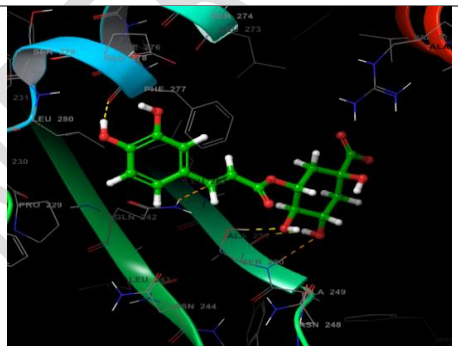
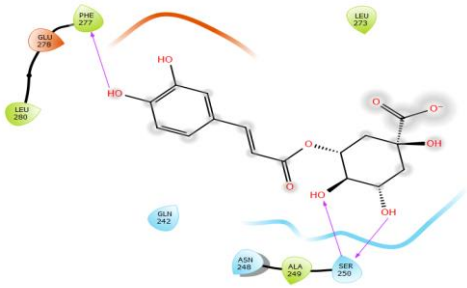
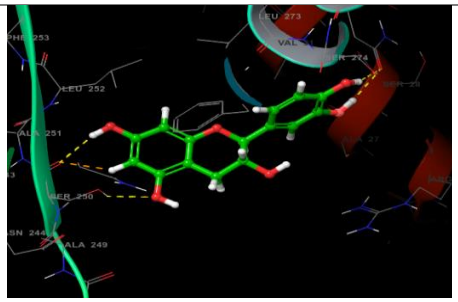
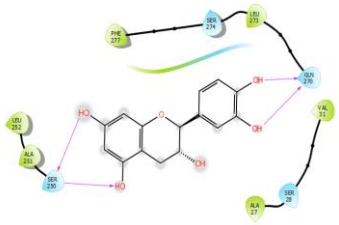
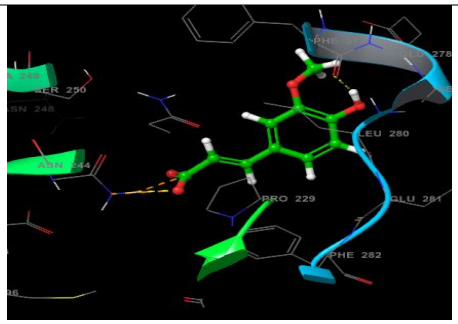
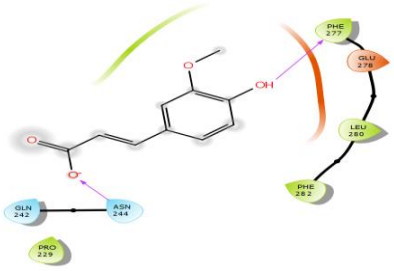
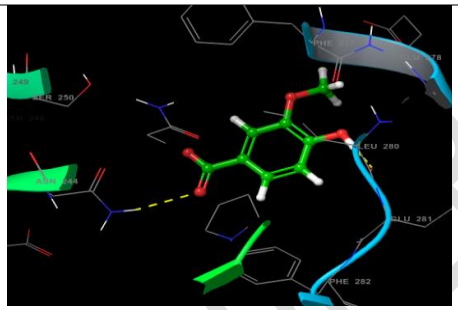
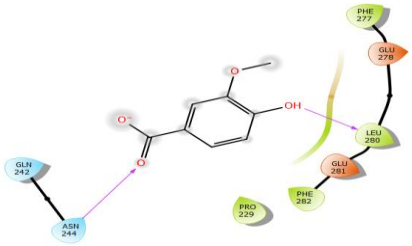
Ferulic acid		

Table 6 : Interaction images of all molecules with Alpha1-antichymotrypsin variant DBS-II: a drug-binding serpin for doxorubicin (PDB ID: 5OM7)

Compound Name	**3D Image**	**2D Image**
Reference Doxorubicin		
Chlorogenic acid		

Catechin hydrate		
Ferulic acid		
Vanillic acid		

3.5 ADMET Analysis:

The ADME properties were assessed using SwissADME. Absorption, distribution, metabolism, and excretion (ADME) characteristics of the compounds were analysed using SwissADME in an attempt to assess the compounds' therapeutic potential. This analysis provided a comprehensive picture of the compounds' drug-likeness and pharmacokinetic profile with respect to significant factors like molecular weight, gastrointestinal absorption capacity, synthetic accessibility, compliance with the "Rule of Five" for oral bioavailability, and the molecule's distribution between fat/water (Log P/o/w) and blood/brain (Log BB). The findings of this investigation will primarily determine if these chemicals are appropriate for

further development as potential medicinal agents. The comprehensive analysis yielded valuable insights into the pharmacological potential of the compounds, enabling the determination of whether more research and development of the molecules as pharmaceuticals were necessary. The complete set of ADME results for SwissADME is shown in Table 7.

Table 7 : ADME prediction (http://www.swissadme.ch/index.php)

Sr. no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1	Chlorogenic acid	354.31 g/mol	9	6	0.96	Low	No	0.11	1	4.16
2	Catechin hydrate	308.28 g/mol	7	6	1.17	High	No	0.55	1	3.60
3	Reference Doxorubicin	543.52 g/mol	12	6	2.16	Low	No	0.17	3	5.81
4	Ferulic acid	194.18 g/mol	4	2	1.62	High	Yes	0.85	0	1.93
5	Vanillic acid	168.15 g/mol	4	2	1.40	High	No	0.85	0	1.42

Utilising MM-GBSA and docking studies, the most promising compounds were identified, and their safety profile was examined using ProTox-II, an online system that predicted probable toxicity. Some of the toxicity outcomes that were investigated in this study were hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity. All of the results of our in-silico toxicity prediction are shown in Table 8.

Table 8: Toxicity study of molecules by ProTox-II

Sr. no.	Compound name	ProTox-II Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	chlorogenic acid	5	Inactive	Less Inactive	Active	Inactive	Inactive
2	catechin hydrate	6	Inactive	Inactive	Inactive	Less Active	Inactive
3	vanillic acid	4	Less Inactive	Less Inactive	Inactive	Inactive	Inactive
4	ferulic acid	4	Less Inactive	Less Inactive	Active	Inactive	Inactive
5	doxorubicin	3	Inactive	Inactive	Active	Active	Active

4. DISCUSSION

There are significant details on the efficiency of ligand-target protein interactions. Higher negative values signify more advantageous interactions and indicate the possibility of these compounds successfully modifying the activities of the target proteins linked to cancer development. This increases the likelihood that they will be desirable subjects for subsequent

treatment-related studies. Apart from the potential anticancer effects of the pharmaceuticals, the ADMET study offers a comprehensive understanding of their pharmacokinetic and toxicological attributes. Vanillic acid, ferulic acid, chlorogenic acid, and catechin hydrate all have different levels of pharmacokinetic properties in their class 5, 4, 6, and 4 profiles. Interestingly, these chemicals have favourable profiles in terms of carcinogenicity and hepatotoxicity, showing no signs of these negative effects. The absence of serious adverse effects in all four compounds, including the reference medication doxorubicin, underscores their suitability for use in pharmaceutical applications. Collectively important results from these studies show that ferulic acid, vanillic acid, chlorogenic acid, and catechin hydrate can be used as medicines to help fight cancer. They are appealing prospects for further preclinical and clinical investigation due to their more advantageous interactions with target proteins, as well as their more effective pharmacokinetic and toxicological properties. These compounds' various properties could lead to the development of less harmful and more effective new anticancer therapies, thereby improving cancer therapy and patient care.

5. CONCLUSION

The docking study of chlorogenic acid, vanillic acid, catechin hydrate, and ferulic acid shows that they bind to anticancer target proteins more strongly than their reference standards. Additionally, ADMET studies of all four compounds have shown that they are useful in pharmaceutical applications. However, additional in vivo and in vitro studies are required to thoroughly assess the therapeutic potential of these drugs.

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