Analysis of plant-derived phytochemicals as anti-cancer agents targeting cyclin dependent kinase-2, human topoisomerase IIα and vascular endothelial growth factor receptor-2

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ABSTRACT
Cancer is caused by a variety of pathways, involving numerous types of enzymes. Among them three enzymes i.e. Cyclin-dependent kinase-2 (CDK-2), Human topoisomerase IIα, and Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) are three of the most common enzymes that are involved in the cancer development. Although many chemical drugs are already available in the market for cancer treatment, plant sources are known to contain a wide variety of agents that are proved to possess potential anticancer activity. In this experiment, total thirty phytochemicals were analyzed against the mentioned three enzymes using different tools of bioinformatics and in silico biology like molecular docking study, drug likeness property experiment, ADME/T test, PASS prediction, and P450 site of metabolism prediction as well as DFT calculation to determine the three best ligands among them that have the capability to inhibit the mentioned enzymes. From the experiment, Epigallocatechin gallate was found to be the best ligand to inhibit CDK-2, Daidzein showed the best inhibitory activities towards the Human topoisomerase IIα, and Quercetin was predicted to be the best agent against VEGFR-2. They were also predicted to be quite safe and effective agents to treat cancer. However, more in vivo and in vitro analyses are required to finally confirm their safety and efficacy in this regard.

1. Introduction
Cancer is defined as the uncontrolled proliferation and abnormal spread of the body’s specific cells. According to WHO, cancer was responsible for 13% of world deaths accounted in 2005. Moreover, projections have shown that cause-specific years of life lost (YLL) rate due to cancer has already increased in 2005 and 2015, and it will continue to be rising in the future. Millions of species of plants, animals, marine organisms and microorganisms act as attractive sources for new therapeutic candidate compounds. However, the development of novel agents from natural sources faces many obstacles that are not usually met when one deals with synthetic compounds. Moreover, there may be difficulties with identification, isolation, assessing, and obtaining the appropriate amounts of the active compounds in the sample [1,2]. The search for anti-cancer compounds from plant sources started in the 1950s with the discovery and development of the various natural compounds like vinca alkaloids, vinblastine, vincristine, and cytotoxic podophyllotoxins. In the recent years, new technologies have been developed by the scientists to enhance natural product drug discovery in an industrial manner. Indeed, several new anticancer agents of natural origin have been introduced to the market recently and there is a promising pipeline of natural products in cancer-related clinical trials [3–6]. Future advances in the directed biosynthesis of small molecules will improve the ability of the scientists to control the shape and topology of various small molecules and thus creating new anti-cancer compounds that will interact specifically with biological targets. In the future, plants (300,000–500,000 such species) will continue to be a vital and valuable resource for anticancer drug discovery. More than 60 compounds from different plant sources are currently in the pipeline as potential anti-cancer agents [7–10]. Many chemical and synthetic drugs are already available for treating cancers i.e. alvocidib, lenvatinib, and daunorubicin etc. These chemical drugs have many adverse effects like sepsis, diarrhea, stomach and bladder pain, hair loss, paralysis, joint pain etc. However, plant phytochemicals are considered as safe in this regard since they generally do not possess any adverse effect to the human health in appropriate doses [11–14]. Therefore, using alternatives from plants can have great potential for cancer...
treatment. Table 1 lists the potential phytochemicals used in the experiment.

### 1.1. Role of cyclin-dependent kinase-2 (CDK-2) in cyclin/CDK pathway and its involvement in cancer

Cyclin/CDK pathway is one of the major cell cycle regulatory pathways, involving the cyclin-dependent kinases (CDKs), Retinoblastoma (Rb) tumor suppressor family, and a family of transcription factors known as E2F. All these components of the pathway are essential for the passage of cells through the G1 to the S phase of the cell cycle. The CDK proteins are serine/threonine kinase that phosphorylate and thus inactivate the Rb protein. In the resting state of cell, Rb inhibits the activity of E2F protein forming a complex with it. The cyclin proteins can be of D type (cyclin D) and E type (cyclin E). Upon activation by the growth-promoting signals or several mitogens, the cyclin D is found to form complex with CDK-4 and CDK-6. However, the cyclin E is found to be associated with CDK-2, when it is activated by active E2F. The cyclin D-CDK-4/6 and cyclin E-CDK-2 complexes phosphorylate and thereby inactivate the Rb protein. This inactivation causes the release of bound E2F transcription factor from the Rb protein. The released E2F later takes part in cell cycle progression. Moreover, E2F also promotes the activation of Cyclin E-CDK-2 complex, which in turn phosphorylates Rb protein and activate E2F transcription factor by feedback loop. Many inhibitors of the CDK proteins also takes part to regulate the cell cycle properly. The inhibitors repress the CDK proteins when there is no need for the cells to divide [68–74]. The inhibitors are proteins from inhibitors of CDK-4 (INK4) and cyclin-dependent kinase inhibitor (CKI) families. CDK-4/6 is inhibited by p15/16 inhibitors and CDK-2 is inhibited by p21/p27 inhibitors. However, any type of mutation in the CDK genes causing hyperactivity or any type of mutation in the inhibitory genes, may lead to the uncontrolled proliferation of the cells, which can lead to different forms of cancers [75]. Therefore, targeting and inhibition of CDK-2 can be a potential strategy for anticancer drug development [76] (Figure 1).

### 1.2. The DNA topoisomerase IIα pathway and its involvement in cancer

Due to the supercoiled structure of the DNA molecules, it is necessary to unwind the double-stranded DNA before replication, transcription, recombination, and other processes. DNA topoisomerases are the enzymes that function in unwinding, cutting, shuffling, and relagating the DNA double helix structure. The human genome encodes six topoisomerases that are grouped into three types: type Iα, type Iβ, and type IIα. DNA topoisomerase IIα is one of the necessary topoisomerases that function in various cellular functions. However, it is a genotoxic enzyme which can lead to cancer development. When DNA topoisomerase II cuts the double-stranded DNA, it may remain covalently attached to the

### Table 1. List of the plant-derived anti-cancer agents that work by targeting the CDK-2, human topoisomerase IIα, and VEGFR-2 pathways.

| Sl. No. | Name of the compounds | PubChem CID | IC50 Value (in μM) | References |
|--------|-----------------------|-------------|--------------------|------------|
| 01     | Geraniol              | 637566      | 20                 | [15,16]    |
| 02     | Epigallocatechin gallate | 65064      | 26                 | [17,18]    |
| 03     | Indirubin             | 10177       | 7.5                | [19–21]    |
| 04     | Fisetin               | 5281614     | 52                 | [22–24]    |
| 05     | Apigenin              | 5280443     | 216.4              | [22,25]    |
| 06     | Luteolin              | 5280445     | 258                | [22,26,27] |
| 07     | Kaempferol            | 5280863     | 28                 | [22,28,29] |
| 08     | Chrysin               | 5281607     | 49.2               | [22,30,31] |
| 09     | Elenoside             | 1,04,58,570 | NA                 | [32]       |
| 10     | Genistein             | 5280961     | 40                 | [22,33,34] |
| 11     | Amentoflavone         | 5281600     | 26                 | [35]       |
| 12     | Cryptolepine          | 82143       | 130                | [36,37]    |
| 13     | Neocryptolepine       | 390526      | 12.7               | [38,39]    |
| 14     | Bakuchin              | 3083848     | 404                | [40]       |
| 15     | Lunacridine           | 442920      | 600                | [41]       |
| 16     | Daidzein              | 5281708     | 78.74              | [42,43]    |
| 17     | Camptothecin          | 24360       | 10.3               | [44,45]    |
| 18     | Salvicine             | 10359290    | 1866               | [46,47]    |
| 19     | Sauchinone            | 11725801    | 29                 | [48]       |
| 20     | Nectandrin B          | 156517      | 12                 | [48]       |
| 21     | Ellagic acid          | 5281855     | 5                  | [49,50]    |
| 22     | Dioscin               | 119245      | 7.36               | [51,52]    |
| 23     | 12-Deoxyxphorbul 13-palmitate | 322885 | 38               | [53,54]    |
| 24     | Melatonin             | 896         | 5000               | [55,56]    |
| 25     | Pristimerin           | 159516      | 16                 | [57,58]    |
| 26     | α-santalol            | 11085337    | 12.34              | [59]       |
| 27     | Plumbagin             | 10205       | 50                 | [60]       |
| 28     | Decursin              | 442126      | 2500               | [61–63]    |
| 29     | Decursinol            | 442127      | 2500               | [61,63,64] |
| 30     | Quercetin             | 5280343     | 10.26              | [65–67]    |

NA: not available.
broken end of the DNA. This reaction intermediate is known also as the cleavage complex. If the amount of the cleavage complex in the cell falls too much, then the cells are not able to divide into daughter cells due to mitotic failure, which results in the death of the cells. Moreover, if the amount of the cleavage complex increases too much, the temporary cleavage complex structures can become permanent double-stranded breaks in the DNA. These double-stranded breaks are caused by the faulty DNA tracking system which then initiates the faulty recombination and repair pathways of DNA replication and expression, leading to cancer (Figure 2). For this reason, DNA topoisomerase IIα is a potential target for anti-cancer drug development [77–81].

1.3. The role of vascular endothelial growth factor receptor-2 (VEGFR-2) in angiogenesis pathway and its involvement in cancer

Angiogenesis is the process of generating new capillary blood vessels [82]. It plays important functions in organ development and differentiation during embryogenesis as well as wound healing and reproductive functions. However, angiogenesis is also responsible for a number of disorders including tumor formation, cancers, rheumatoid arthritis etc. Vascular Endothelial Growth Factor (VEGF) plays key role in angiogenesis process. VEGF protein has many isoforms and all of the isoforms mediate their effects by specific receptors known as VEGF receptors (VEGFRs). VEGFRs are receptor tyrosine kinases (RTKs) and there are three main isoforms: VEGFR-1, VEGFR-2, and VEGFR-3. The expression of VEGF protein is found to be dramatically increased in cancers like lung, thyroid, breast, ovary, kidney, uterine cancers etc. [83,84]. Since VEGF mediates its effects by binding to specific receptors (like VEGFR-2), inhibiting the actions of the receptors is thought to be a therapeutic target for cancer treatment [85]. When VEGF protein binds with VEGFR-2, the VEGFR-2 becomes activated which then activates phosphatidylinositol 3-kinase (PI3K). PI3K further activates phosphoinositide-3-kinase (PIρ3), which in turn activates the Akt/PKB (protein kinase B) signaling pathway. This pathway contributes to endothelial cell survival by activating proteins, like BAD (Bcl-2 associated death promoter) and caspase proteins. Moreover, the Akt/PKB signaling pathway can activate the endothelial nitric-oxide synthase (eNOS), which is responsible for vascular permeability. Both the endothelial cell survival and vascular permeability mechanisms contribute to the angiogenesis process. The binding of VEGF to VEGFR-2 can sometimes activate MAP kinase (mitogen activated protein kinase) pathway which is responsible for the proliferation of endothelial cells. In this pathway, activated VEGFR-2 activates

Figure 1. The cyclin/CDK signaling pathway. Upon activated by mitogen signal, the cyclin D-CDK-4/6 complex is activated and cause the inactivation of Rb by phosphorylation and thus release the active E2F, which takes part in cell cycle progression. However, E2F activates cyclin E-CDK-2 complex, which phosphorylates the Rb protein and activates the E2F in a feedback loop. p15/p16 inhibitors repress cyclin D-CDK-4/6 complex and p21/p27 inhibit cyclin E-CDK-2. Anti-CDK-2 agents inhibit the CDK-2 protein, thus can help in cancer treatment.
phospholipase c-\(\gamma\) (PLC-\(\gamma\)). The PLC-\(\gamma\) then activates the protein kinase C (PKC), which further activates the proteins of MAP kinase pathway: RAF1, MEK, ERK, sequentially. This MAP kinase pathway causes the endothelial cell proliferation, which also contributes to the angiogenesis process (Figure 3) \([86–88]\). Since VEGFR-2 is involved in angiogenesis process in cancer development, inhibition of VEGFR-2 is considered as therapeutic approach to treat cancer.

Three approved drugs were used as positive controls in this study: alvocidib (inhibits CDK-2), daunorubicin (inhibits human topoisomerase II\(\alpha\)), and lenvatinib (inhibits VEGFR-2) \([32,89,90]\).

2. Materials and methods

Ten ligands (total) for each of the target molecule i.e. CDK-2, human topoisomerase II\(\alpha\), and VEGFR-2, were selected that have already been proven to have inhibitory effects on cancer. Their IC50 values were collected by reviewing literatures discussing their anticancer potentiality. On sequential docking experiment one best ligand molecule was selected as the best inhibitor of respective target. Then their different drug-like parameters were analyzed in different experiments.

2.1. Protein preparation and Ramachandran plot generation

Three-dimensional structures of Cyclin-dependent kinase-2 (3EZV), Human topoisomerase II (1ZXM), and Vascular Endothelial Growth Factor Receptor-2 (2OH4) were downloaded (sequentially) in PDB format from protein data bank (www.rcsb.org). The proteins were then prepared and refined using the Protein Preparation Wizard in Maestro Schrödinger Suite 2018-4 \([91]\). Bond orders were assigned and hydrogen molecules were added to heavy atoms as well as all the waters were deleted and the side chains were adjusted using Prime \([92]\). Finally, the structure was optimized and then minimized using force field OPLS_2005. Minimization was done setting the maximum heavy atom RMSD (root-mean-square-deviation) to 30 Å and any remaining water less than 3 H-bonds to non-water was again deleted during the minimization step. After successful minimization, the proteins were used to generate Ramachandran plots for each of the protein by Maestro Schrödinger Suite 2018-4, keeping all the parameters as default.

2.2. Ligand preparation

Three-dimensional structures of 30 selected ligand molecules as well as controls were downloaded (sequentially) from PubChem database (www.pubchem.ncbi.nlm.nih.gov). These structures were then prepared using the LigPrep function of Maestro Schrödinger Suite \([93]\). Minimized 3D structures of ligands were generated using Epik2.2 and within pH 7.0 ± 2.0 \([94]\). Minimization was again carried out using OPLS_2005 force field which generated 32 possible stereoisomers.

Figure 2. The DNA topoisomerase II\(\alpha\) pathway in cancer development. Upon the cleavage of the target DNA, the topoisomerase can remain bound to the cleaved ends of the DNA fragments and form cleavage complexes. If the concentration of cleavage complexes falls too much, then this may lead to cell death due to mitotic failure. Moreover, if the concentration rises too much, abnormal translocations and mutagenesis may occur, which lead to cancer development. Anti-topoisomerase agents aid in cancer treatment by inhibiting the activity of DNA topoisomerase II\(\alpha\).
2.3. Receptor grid generation

Grid usually confines the active site to shortened specific area of the receptor protein for the ligand to dock specifically. In Glide, a grid was generated using default Van der Waals radius scaling factor 1.0 and charge cutoff 0.25 which was then subjected to OPLS_2005 force field. A cubic box was generated around the active site (reference ligand active site). Then the grid box volume was adjusted to $15 \times 15 \times 15$ for docking test.

2.4. Glide standard precision (SP) ligand docking, prime MM-GBSA calculation, and induced fit docking

SP adaptable glide docking was carried out using Glide in Maestro Schrödinger Suite [95]. The Van der Waals radius scaling factor and charge cutoff were set to 0.80 and 0.15 respectively for all the ligand molecules. Final score was assigned according to the pose of docked ligand within the active site of the receptor.

This technique utilizes the docked complex and uses an implicit solvent which then assigns more accurate scoring function and improves the overall free binding affinity score upon the reprocessing of the complex. It combines OPLS molecular mechanics energies ($E_{MM}$), surface generalized born solvation model for polar solvation ($G_{SGB}$), and a nonpolar salvation term ($G_{NP}$) for total free energy ($\Delta G_{bind}$) calculation. The total free energy of binding was calculated by the following equation: [96]

$$\Delta G_{bind} = G_{complex} - (G_{protein} - G_{ligand})$$

where,

$$G = E_{MM} + G_{SGB} + G_{NP}.$$ 

Nine anticancer agents were selected on the basis of best MM-GBSA scores.

At this stage the docking parameters of our compounds under investigation was compared with three controls name with respective receptors.

To carry out the IFD of the nine selected ligand molecules, again OPLS_2005 force field was applied after generating grid around the co-crystallized ligand of the receptor. Receptor and Ligand Van Der Waals screening parameters were set at 0.70 and 0.50 respectively and residues within 2 Å were refined to generate 2 best possible poses with extra precision. The best performing ligand from each enzyme category was selected according to the IFD score.

Figure 3. The angiogenesis pathway. The VEGF protein binds with VEGFR-2 and activates the receptor. The VEGFR-2 activates PI3K, which activates PI3P and thus activating the Akt/PKB signaling pathway. This pathway contributes to endothelial cell survival by activating BAD and caspase proteins. Moreover, the Akt/PKB signaling pathway can activate eNOS, which is responsible for vascular permeability. Both the endothelial cell survival and vascular permeability mechanisms contribute to the angiogenesis process. Binding of VEGF to VEGFR-2 can sometimes activate MAP kinase pathway which is responsible for the proliferation of endothelial cells. The activated VEGFR-2 activates PLC-γ. The PLC-γ further activates PKC. PKC further activates RAF1, MEK, ERK, sequentially. This MAP kinase pathway causes the endothelial cell proliferation, which also contributes to the angiogenesis process. VEGFR-2 inhibitors inhibit VEGFR-2, thus aid in cancer treatment.
and XP_Gscore. The 3D representations of the best pose interactions between the ligands and their respective receptors were obtained using Discovery Studio Visualizer [97]. After the docking analysis, the plot depicting the relationship between the docking scores and IC50 values, was generated. For generating the plot, the IC50 values were converted to the log10 (IC50) values. Then the docking scores were placed at the X-axis and the log10 (IC50) values were put on the Y-axis for generating the relationship plot.

2.5. Ligand-based drug-likeness property analysis and ADME/toxicity prediction

The drug-likeness properties of the three selected ligand molecules were analyzed using SWISSADME server (http://www.swissadme.ch/) [98]. The ADME/T for each of the ligand molecules was carried out using online-based servers, admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) and ADMETlab (http://admet.scbdd.com/) to predict their various pharmacokinetic and pharmacodynamic properties [99,100]. The absorption, distribution, and metabolism properties were determined by both admetSAR server and excretion and toxicity properties were determined by ADMETlab server. The numeric and categorical values of the results given by ADMETlab server were changed into qualitative values according to the explanation and interpretation described in the ADMETlab server (http://admet.scbdd.com/home/interpretation/) for the convenience of interpretation.

2.6. PASS (prediction of activity spectra for substances) and P450 site of metabolism (SOM) prediction

The PASS (Prediction of Activity Spectra for Substances) prediction of the three best-selected ligands was conducted by using PASS-Way2Drug server (http://www.pharmaexpert.ru/passonline/) by using canonical SMILES from PubChem server (https://pubchem.ncbi.nlm.nih.gov/) [101]. To carry out PASS prediction, Pₐ (probability ‘to be active’) was kept greater than 70%, since the Pₐ > 70% threshold gives highly reliable prediction [102]. In the PASS prediction study, both the possible biological activities and the possible adverse effects of the selected ligands were predicted. The P450 Site of Metabolism (SOM) of the three best-selected ligand molecules was determined by an online tool, RS-WebPredictor 1.0 (http://reccr.chem.rpi.edu/Software/RS-WebPredictor/) [103]. The LD50 and Toxicity class were predicted using ProTox-II server (http://tox.charite.de/protox_II/) [104].

2.7. DFT calculations

Minimized ligand structures obtained from LigPrep were used for DFT calculation using the Jaguar panel of Maestro Schrödinger Suite using Becke’s three-parameter exchange potential and Lee-Yang-Parr correlation functional (B3LYP) theory with 6-31G* basis set [105–107]. Quantum chemical properties such as surface properties (MO, density, potential) and Multipole moments were calculated along with HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy. Then the global frontier orbital was analyzed and hardness (η) and softness (S) of selected molecules were calculated using the following equation as per Parr and Pearson interpretation and Koopmans theorem [108,109]. The DFT calculation was done for the three best ligand molecules.

\[ \eta = \frac{(\text{HOMO} - \text{LUMO})}{2}, \quad S = \frac{1}{\eta} \]

3. Results

3.1. Ramachandran plot and molecular docking analysis

After preparing the proteins, the Ramachandran plot for each of the receptor proteins was generated. In the plot, the orange regions represent ‘favored’ regions, the yellow regions represent ‘allowed’ regions, and the white regions represent ‘disallowed’ regions [110]. CDK-2 protein generated Ramachandran plot with almost all of the amino acids in the ‘favored’ region and no amino acids in the ‘disallowed region’. Human topoisomerase II generated Ramachandran plot with 15 amino acids in the ‘disallowed region’. It also had majority of the amino acids in the ‘favored’ region. VEGFR-2 generated Ramachandran plot with only four amino acids in the ‘disallowed region’ and most of the amino acids in the ‘favored’ region (Figure 4).

All the selected ligand molecules were docked successfully with their respective receptor proteins. The ligand molecules that had the lowest binding energy were considered the best ligand molecules in inhibiting their respective receptors since lower binding energy (docking score) corresponds to higher binding affinity [111]. In the MM-GBSA study, the most negative ΔG-bind score (the lowest score) is considered as the best ΔG-bind score [112]. IFD study is carried out to understand the accurate binding mode and to ensure the accuracy of active site geometry. The lowest values of IFD score and XP Gscore are considered as the best values [113–116]. Nine ligands: Geraniol, Epigallocatechin gallate, and Indirubin (inhibit CDK-2), Daidzein, Camptothecin, and Salvicine (inhibit human topoisomerase II) and Quercetin, Ellagic acid, and Plumbagin (inhibit VEGFR-2), were initially selected based on the lower free binding energy and MM-GBSA study since they were reported to show comparable binding energy with respective controls (Table 2). Then these molecules were subjected to IFD study. Epigallocatechin gallate, Daidzein, and Quercetin were considered as the three best ligand molecules from the IFD study among the nine initially selected ligands. The 3D representations as well as interaction of different amino acids with Epigallocatechin gallate, Daidzein, and Quercetin are illustrated in Figure 5.

Now, these three best ligands (one from each of the receptor category) were used in next phases of this experiment to analyze their drug potentials.

3.1.1. Binding mode of best ligands with their respective targets

Epigallocatechin gallate docked with CDK-2 with an IFD score of −594.995 Kcal/mol and XP Gscore of −8.816 Kcal/mol. It formed
Figure 4. Ramachandran plot analysis of (1) CDK-2, (2) Human topoisomerase II, (3) VEGFR-2. Glycine and proline are represented as triangles and squares and all other amino acids are represented as spheres.

Table 2. The results of molecular docking study between the selected 30 ligands and their receptors.

| No | Name of ligand | Name of receptor | Docking score/binding energy (Kcal/mol) | Glide energy (Kcal/mol) | MM-GBSA (ΔGbind Score Kcal/mol) |
|----|----------------|------------------|-----------------------------------------|-------------------------|----------------------------------|
| Control-1 | Alvocidib | CDK-2 (PDB ID: 3EZV) | -5.144 | -42.707 | -71.530 |
| 01 | Geraniol | | -7.341 | -48.430 | -59.370 |
| 02 | Epigallocatechin gallate | | -7.123 | -60.544 | -66.420 |
| 03 | Indirubin | | -8.410 | -33.776 | -53.960 |
| 04 | Fisetin | | -3.836 | -42.105 | -37.819 |
| 05 | Apigenin | | 3.836 | 36.499 | 44.342 |
| 06 | Luteolin | | 4.954 | 38.551 | 32.109 |
| 07 | Chrysin | | 6.893 | 34.446 | 46.700 |
| 08 | Elenoside | | -5.445 | -46.435 | -35.451 |
| 09 | Genistein | | -6.119 | -37.710 | -31.310 |
| Control-2 | Daunorubicin | Human topoisomerase II | -5.469 | -39.191 | -40.326 |
| 11 | Amentoflavone | (PDB ID: 1ZXM) | -5.524 | -32.638 | -36.549 |
| 12 | Cryptolepine | | -5.802 | -40.963 | -22.341 |
| 13 | Neocryptolepine | | -6.177 | -39.058 | -37.330 |
| 14 | Bakuchicin | | -4.638 | -37.756 | -40.004 |
| 15 | Lunacridine | | -5.413 | -42.872 | -21.934 |
| 16 | Daizein | | -7.855 | -42.546 | -55.980 |
| 17 | Camptothecin | | -7.630 | -48.500 | -40.223 |
| 18 | Salvicine | | -6.969 | -42.072 | -44.550 |
| 19 | Sauchinone | | -6.266 | -42.390 | -34.449 |
| 20 | Nectandrin B | | -6.173 | -43.608 | -32.870 |
| Control-3 | Lenvatinib | VEGFR-2 (PDB ID: 2OH4) | -9.745 | -61.045 | -70.240 |
| 21 | Ellagic acid | | -7.039 | -44.384 | -46.776 |
| 22 | Dioscin | | -4.524 | -33.341 | -32.200 |
| 23 | 12-Deoxyphorbol | 13-palmitate | -6.471 | -47.617 | -32.239 |
| 24 | Melatonin | | -3.996 | -36.512 | -46.450 |
| 25 | Pristimerin | | -6.179 | -37.520 | -33.984 |
| 26 | α-santalol | | -6.494 | -33.456 | -41.230 |
| 27 | Plumbagin | | -7.848 | -40.639 | -48.910 |
| 28 | Decursin | | -6.307 | -38.690 | -21.430 |
| 29 | Decursinol | | -8.960 | -49.149 | -59.710 |
| 30 | Quercetin | | -10.441 | -54.972 | -64.420 |
six conventional hydrogen bonds with Lysine 89, Leucine 298, Histidine 84, Glutamic acid 08, Leucine 134, and Glutamine 131 (×2) at 1.82 Å, 1.53 Å, 2.13 Å, 1.55 Å, 4.53 Å, 1.69 Å, and 2.45 Å distance apart respectively within the binding pocket of CDK-2. Moreover, it also formed one non-conventional hydrogen bond with Histidine 84. Epigallocatechin gallate was also reported to

Figure 5. Best possible poses (left) and 2D interactions (right) between ligand and receptor molecules.
form multiple hydrophobic interactions i.e. Pi-Alkyl with Isoleucine 10 and Leucine 34 amino acid residues within the binding cleft of CDK-2 (Table 3).

Daidzein docked with Topoisomerase IIa with an IFD score of −730.514 Kcal/mol and XP Gscore of −8.152 Kcal/mol. It formed four conventional hydrogen bonds with Asparagine 120, Threonine 215, and Isoleucine 125, at 1.76 Å, 2.46 Å, and 4.79 Å distance apart respectively within the binding cleft of CDK-2. Daidzein was also reported to form multiple hydrophobic interactions i.e. Pi-Alkyl with Isoleucine 125 (×2) and Alanine 167 amino acid residues within the binding pocket of Human topoisomerase IIa (Table 3).

Quercetin (vascular endothelial growth factor receptor-2) docked with VEGFR-2 with an IFD score of −675.939 Kcal/mol and XP Gscore of −12.030 Kcal/mol. It formed six conventional hydrogen bonds with Glutamic acid 883, Aspartic acid 1044, and Alanine 864, at 2.69 Å, 2.12 Å, and 4.05 Å distance apart respectively within the binding cleft of CDK-2. It also formed a non-conventional hydrogen bond with Phenylalanine 916 at 2.51 Å distance. It was also reported to form multiple hydrophobic interactions i.e. Pi-Alkyl with Leucine 838, Valine 914, and many other amino acid residues within the binding pocket of VEGFR-2 (Table 3).

After the docking analysis, the relationship plot of docking scores vs log10 (IC50) values (in relationship to the MM-GBSA scores), was generated. From the plot, it can be concluded that most of the docking scores were predicted to have quite good relationship with the log10 (IC50) values as the lower docking scores had relationship with lower log10 (IC50) values or just the IC50 values and vice versa. Although, such relational plots do not always express the one to one relationship of docking scores and IC50 values, however, in our study we have found out quite substantial relationship among them (Supplementary Table S1 and Supplementary Figure S1).

### 3.2. Drug-likeness properties

Among the three ligand molecules, only Epigallocatechin gallate violated the Lipinski’s rule of five (2 violations: number of hydrogen bond donors and acceptors). However, it

| Name of the ligand (with respective receptor) | XP GScore (Kcal/mol) | IFD score (Kcal/mol) | Interacting amino acids | Bond distance in Å | Interaction category | Type of interaction |
|-----------------------------------------------|----------------------|----------------------|-------------------------|-------------------|---------------------|---------------------|
| Epigallocatechin gallate (cyclin-dependent kinase-2) | −8.816 | −594.995 | Lysine 89 | 1.82 | Hydrogen bond | Conventional |
| | | | Leucine 298 | 1.53 | Hydrogen bond | Conventional |
| | | | Histidine 84 | 2.13 | Hydrogen bond | Conventional |
| | | | Glutamic acid 08 | 1.53 | Hydrogen bond | Conventional |
| | | | Isoleucine 10 | 4.84 | Hydrophobic bond | Pi-Alkyl |
| | | | Leucine 134 | 4.53 | Hydrophobic bond | Pi-Alkyl |
| | | | Glutamine 131 | 1.69 | Hydrogen bond | Conventional |
| | | | Aspartic acid 1044 | 2.80 | Hydrogen bond | Conventional |
| | | | Cysteine 1043 | 5.76 | Miscellaneous | Pi-stacked |
| | | | Phenylalanine 916 | 2.51 | Hydrogen bond | Conventional |
| | | | Glutamic acid 883 | 2.69 | Hydrogen bond | Conventional |
| | | | Lysine 866 | 5.30 | Hydrophobic bond | Pi-Alkyl |
| | | | Valine 914 | 4.81 | Hydrophobic bond | Pi-Alkyl |
| | | | Phenylalanine 916 | 2.51 | Hydrogen bond | Conventional |
| | | | Threonine 215 | 2.46 | Hydrogen bond | Conventional |
| | | | Isoleucine 125 | 4.79 | Hydrophobic bond | Pi-Alkyl |
| | | | Asparagine 91 | 2.96 | Hydrogen bond | Conventional |
| | | | Alanine 167 | 4.88 | Hydrophobic bond | Pi-Alkyl |
| | | | Phenylalanine 142 | 5.59 | Hydrophobic bond | Pi-Pi T-shaped |
| | | | Lysine 168 | 2.65 | Hydrogen bond | Conventional |
| | | | Isoleucine 141 | 4.68 | Hydrophobic bond | Pi-Alkyl |
| Quercetin (vascular endothelial growth factor receptor-2) | −12.030 | −675.939 | Asparagine 120 | 1.76 | Hydrogen bond | Conventional |
| | | | Threonine 215 | 2.46 | Hydrogen bond | Conventional |
| | | | Isoleucine 125 | 4.79 | Hydrophobic bond | Pi-Alkyl |
| | | | Asparagine 91 | 2.96 | Hydrogen bond | Conventional |
| | | | Valine 914 | 4.81 | Hydrophobic bond | Pi-Alkyl |
| | | | Glutamic acid 915 | 2.12 | Hydrogen bond | Conventional |
| | | | Phenylalanine 916 | 2.51 | Hydrogen bond | Conventional |
| | | | Alanine 864 | 4.05 | Hydrophobic bond | Pi-Alkyl |
| | | | Leucine 1033 | 5.32 | Hydrophobic bond | Pi-Alkyl |
| | | | Cysteine 917 | 2.39 | Hydrogen bond | Conventional |
| | | | Leucine 1033 | 5.32 | Hydrophobic bond | Pi-Alkyl |
| | | | Cysteine 1043 | 5.76 | Miscellaneous | Pi-Sulfur |
| | | | Aspartic acid 1044 | 2.80 | Hydrogen bond | Conventional |
| | | | Phenylnalanine 1045 | 5.17 | Hydrophobic bond | Pi-Pi stacked |
| | | | Phenylalanine 916 | 2.51 | Hydrogen bond | Conventional |
| | | | Alanine 864 | 4.05 | Hydrophobic bond | Pi-Alkyl |
| | | | Valine 846 | 4.49 | Hydrophobic bond | Pi-Alkyl |
| | | | Leucine 838 | 1.72 | Hydrogen bond | Conventional |
| | | | Valine 914 | 4.81 | Hydrophobic bond | Pi-Alkyl |
| | | | Glutamic acid 883 | 2.69 | Hydrogen bond | Conventional |
| | | | Lysine 866 | 5.30 | Hydrophobic bond | Pi-Alkyl |
| | | | Glutamic acid 915 | 2.12 | Hydrogen bond | Conventional |
| | | | Phenylnalanine 916 | 2.51 | Hydrogen bond | Conventional |
| | | | Threonine 215 | 2.46 | Hydrogen bond | Conventional |
| | | | Isoleucine 125 | 4.79 | Hydrophobic bond | Pi-Alkyl |
| | | | Asparagine 91 | 2.96 | Hydrogen bond | Conventional |

Table 3. The results of docking studies between the three best ligands and their respective receptors, along with their interaction with different types of amino acids and the bonds formed between the ligands and the amino acids.
showed the highest topological polar surface area (TPSA) value of 197.37 Å² (Table 4). Daidzein was found to have the highest LogP value and again Epigallocatechin Gallate showed highest molar refractivity. Furthermore, both Daidzein and Quercetin each were reported to have one rotatable bond and Epigallocatechin gallate was predicted to have four bonds.

### 3.3. ADME/T tests

The results of ADME/T test with probability scores are summarized in Table 5. In the absorption section, only Daidzein showed positive Caco-2 permeability and all the three selected ligands were HIA positive. In the distribution section, all the molecules showed high capability to bind with plasma protein (PPB), however, all of them were not blood–brain barrier permeable (BBB). In the metabolism section, only Epigallocatechin gallate was not inhibitory to CYP450 1A2 and quercetin was the only inhibitor of CYP450 3A4. None of the ligands were found to be substrate for CYP450 2C9 and CYP450 2D6 and CYP450 2D6 had no predicted inhibitor. In the excretion section, Epigallocatechin gallate, Daidzein, and Quercetin showed a half-life of 1.7, 1.5 and 0.2 h, respectively. Only Epigallocatechin gallate showed hERG blocking capability, however, it did not have any human hepatotoxic activity (H-HT negative). Only Daidzein showed a negative result in the Ames mutagenicity test. However, all of them were DILI positive.

### 3.4. Pass and P450 site of metabolism (SOM) prediction

The predicted LD50 value for Epigallocatechin gallate, Daidzein, and Quercetin was 1000, 2430, and 159 mg/kg, respectively. The prediction of activity spectra for substances (PASS prediction) was for the three selected ligands to identify 20 intended biological activities and 5 adverse and toxic effects. The PASS prediction results of all the three selected ligands are listed in Tables 6 and 7. The possible sites of metabolism by CYPs 1A2, 2A6, 2B6, 2C19, 2C9, 2D6, 2E1, and 3A4 of Epigallocatechin gallate, Daidzein, and Quercetin were determined (Table 8). The possible sites of metabolism by the isoforms are indicated by circles on the chemical structure of the molecule [117].

### 3.6. Analysis of Frontier’s orbitals

In the analysis of Frontier’s orbitals, the DFT calculations and HOMO-LUMO studies were conducted. The results of the DFT calculations are listed in Table 9. In these studies, Epigallocatechin gallate showed the lowest gap energy of 0.070 eV as well as the lowest dipole moment of 1.840 debye. On the other hand, Quercetin generated the highest gap energy of 0.167 eV and the highest dipole moment of 5.289 debye. The order of gap energies and dipole moments of these three compounds was Epigallocatechin gallate < Daidzein < Quercetin (Figure 6).
4. Discussions

Molecular docking is an effective strategy in computer-aided drug designing which works on specific algorithms and assigns affinity scores depending on the poses of ligands inside the binding pocket of specific targets. The lowest docking score reflects the highest affinity, meaning that the complex will remain more time in contact [118,119].

In this study, a total of 30 ligands targeting three macromolecules involved in cancer development were screened with the aid of molecular docking which generated comparable docking score as with positive controls (Table 2). At the initial step, their quality was exemplified with the help of Ramachandran plot where they were predicted to perform well. Primarily, three ligands were selected for each of the receptors which were then subjected to IFD. Finally, Epigallocatechin gallate, Daidzein, and Quercetin were selected as the best inhibitors of CDK-2, Human topoisomerase IIα, and VEGFR-2, respectively. Hydrogen and hydrophobic interactions are important for strengthening the receptor–ligand interactions [120]. The selected best three ligands along with the other ligands were predicted to form multiple hydrogen and hydrophobic interactions with the target molecules (Tables 2 and 3).

Estimation of the drug-likeness properties facilitates the drug discovery and development process. Drug permeability through the biological barrier is influenced by the molecular weight and TPSA. The higher the molecular weight and TPSA, the lower the permeability of the drug molecule is and vice versa. Lipophilicity (expressed as LogP) affects the absorption of the drug molecule in the body and higher LogP associates with lower absorption. The number of hydrogen bond donors and acceptors beyond the acceptable range also affects the capability of a drug molecule to cross the cell membrane. The number of rotatable bonds also affects the drug-likeness properties and the acceptable range is less than 10. Moreover, Lipinski’s rule of five demonstrates that a successful drug molecule should have properties within the acceptable range of the five Lipinski’s rules [121,122]. Daidzein and Quercetin were reported to obey standard rule, whereas, Epigallocatechin gallate was reported to violate the rule which might subject it to further modification (Table 4).

The main purpose of conducting ADME/T tests is to determine the pharmacological and pharmacodynamic properties of a candidate drug molecule within a biological system. Therefore, it is a crucial determinant of the success of a drug discovery expenditure. BBB is the most important factor for those drugs that target primarily the brain cells. P-glycoprotein in the cell membrane aids in transporting many drugs, therefore, its inhibition affects the drug transport. The

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Table 6. The PASS prediction results showing the biological activities of the best three ligand molecules.

| Sl no | Biological activities | Epigallocatechin gallate | Daidzein | Quercetin |
|-------|-----------------------|------------------------|----------|-----------|
|       |                       | Pₐ | Pi        | Pₐ | Pi        | Pₐ | Pi        |
| 01    | Antioxidant           | 0.814 | 0.003 | 0.705 | 0.023 | 0.872 | 0.003 |
| 02    | Reductant             | 0.944 | 0.002 | 0.836 | 0.003 | 0.887 | 0.00014 |
| 03    | Anticarcinogenic      | 0.841 | 0.004 | 0.877 | 0.0014 | 0.973 | 0.002 |
| 04    | Antimutagenic         | 0.809 | 0.015 | 0.706 | 0.007 | 0.760 | 0.007 |
| 05    | Chemopreventive       | 0.950 | 0.003 | 0.937 | 0.004 | 0.844 | 0.008 |
| 06    | Membrane integrity agonist | 0.962 | 0.003 | 0.936 | 0.002 | 0.833 | 0.030 |
| 07    | Hepatoprotectant      | 0.934 | 0.001 | 0.887 | 0.0014 | 0.833 | 0.002 |
| 08    | Mucomembranous protector | 0.856 | 0.003 | 0.756 | 0.002 | 0.969 | 0.002 |
| 09    | Free radical scavenger | 0.879 | 0.007 | 0.765 | 0.002 | 0.909 | 0.001 |
| 12    | APOA1 expression enhancer | 0.977 | 0.005 | 0.909 | 0.003 | 0.970 | 0.003 |
| 13    | Antithrombin activator | 0.941 | 0.003 | 0.941 | 0.003 | 0.909 | 0.003 |
| 14    | Antioxidant           | 0.814 | 0.003 | 0.705 | 0.023 | 0.872 | 0.003 |
| 15    | Oxidoreductase inhibitor | 0.766 | 0.003 | 0.766 | 0.004 | 0.722 | 0.003 |
| 16    | CYP1A1 inhibitor      | 0.771 | 0.003 | 0.771 | 0.004 | 0.722 | 0.003 |
| 17    | CYP1A2 inhibitor      | 0.771 | 0.003 | 0.771 | 0.004 | 0.722 | 0.003 |
| 18    | Antitumor            | 0.771 | 0.003 | 0.771 | 0.004 | 0.722 | 0.003 |
| 19    | Cardioprotectant      | 0.771 | 0.003 | 0.771 | 0.004 | 0.722 | 0.003 |
| 20    | Vasoprotector         | 0.771 | 0.003 | 0.771 | 0.004 | 0.722 | 0.003 |

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Table 7. The PASS prediction results showing the adverse and toxic effects of the best three ligand molecules.

| Sl no | Adverse and toxic effects | Epigallocatechin gallate | Daidzein | Quercetin |
|-------|---------------------------|------------------------|----------|-----------|
|       |                           | Pₐ | Pi        | Pₐ | Pi        | Pₐ | Pi        |
| 01    | Inflammation              | 0.811 | 0.014 | 0.823 | 0.090 | 0.872 | 0.003 |
| 02    | Toxic, vascular           | 0.804 | 0.017 | 0.783 | 0.044 | 0.766 | 0.052 |
| 03    | Twitching                 | 0.790 | 0.027 | 0.772 | 0.03 | 0.783 | 0.052 |
| 04    | Shivering                 | 0.790 | 0.027 | 0.772 | 0.03 | 0.783 | 0.052 |
| 05    | Reproductive dysfunction  | 0.790 | 0.027 | 0.772 | 0.03 | 0.783 | 0.052 |

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Table 8. The P450 site of metabolism prediction of the best three ligand molecules.

| Names of P450 isoenzymes | Epigallocatechin gallate | Daidzein | Quercetin |
|--------------------------|--------------------------|----------|-----------|
| 1A2                      | ![Structure](image1)      | ![Structure](image2) | ![Structure](image3) |
| 2A6                      | ![Structure](image4)      | ![Structure](image5) | ![Structure](image6) |
| 2B6                      | ![Structure](image7)      | ![Structure](image8) | ![Structure](image9) |
| 2C8                      | ![Structure](image10)     | ![Structure](image11) | ![Structure](image12) |
| 2C9                      | ![Structure](image13)     | ![Structure](image14) | ![Structure](image15) |
| 2C19                     | ![Structure](image16)     | ![Structure](image17) | ![Structure](image18) |
| 2D6                      | ![Structure](image19)     | ![Structure](image20) | ![Structure](image21) |
| 2E1                      | ![Structure](image22)     | ![Structure](image23) | ![Structure](image24) |
| 3A4                      | ![Structure](image25)     | ![Structure](image26) | ![Structure](image27) |
permeability of Caco-2 cell line indicates that the drug is easily absorbed in the intestine. Orally absorbed drugs travel through the blood circulation and deposit back to liver and are degraded by a group of enzymes of Cytochrome P450 family and excreted as bile or urine. Therefore, inhibition of any of these enzymes affects the biodegradation of the drug molecule [123,124]. Moreover, if a compound is found to be a substrate for one or more CYP450 enzyme or enzymes, then that compound is metabolized by the respective CYP450 enzyme or enzymes [125]. A drug’s proficiency and pharmacodynamics are depended on the degree of its binding with the plasma protein. A drug can cross the cell layers or diffuse easily if it binds to the plasma proteins less efficiently and vice versa [126]. Human intestinal absorption (HIA) is a crucial process for the orally administered drugs [127–129]. Moreover, the half-life of a drug describes that the greater the half-life, the longer it would stay in the body and the greater its potentiality [130–132]. HERG is a K⁺ channel found in the heart muscle and blocking the hERG signaling can lead to cardiac arrhythmia [133,134]. Human hepatotoxicity (H-HT) involves any type of injury to the liver that may lead to organ failure and death [135,136]. Ames test is a mutagenicity assay that is used to detect the potential mutagenic chemicals [137]. Drug-induced liver injury (DILI) is the injury to the liver that is caused by administration of drugs. DILI is one of the causes that causes acute liver failure [138]. The results of ADME/T test are listed in Table 5. All of the three ligands were predicted to perform similar and sound in the ADME/T test.

Prediction of Activity Spectra for Substances or PASS prediction is a process that is used to estimate the possible profile of biological activities associated with drug-like molecules. Two parameters are used for the PASS prediction: $P_a$ and $P_i$. The $P_a$ is the probability of a compound ‘to be active’ and $P_i$ is the probability of a compound ‘to be inactive’ and their values can range from zero to one [101]. If the value of $P_a$ is greater than 0.7, then the probability of exhibiting the activity of a substance in an experiment is higher [139]. PAS was predicted for Epigallocatechin gallate, Daidzein, and Quercetin. Both Epigallocatechin gallate and Quercetin showed similar and sound performances in the PASS prediction experiment (Tables 6 and 7).

**Table 9.** The results of the DFT calculations of the selected best three ligands.

| Compound name            | HOMO energy (eV) | LUMO energy (eV) | Gap (eV) | Hardness ($\eta$) (eV) | Softness (S) (eV) | Dipole moment (Debye) |
|--------------------------|------------------|------------------|----------|------------------------|------------------|-----------------------|
| Epigallocatechin gallate | 0.050            | 0.120            | 0.070    | 0.035                  | 28.571           | 1.840                 |
| Daidzein                 | –0.040           | 0.040            | 0.080    | 0.040                  | 25.000           | 3.790                 |
| Quercetin                | –0.212           | –0.045           | 0.167    | 0.084                  | 11.904           | 5.289                 |

**Figure 6.** The HOMO and LUMO occupation. (1) Epigallocatechin gallate, (2) Daidzein, and (3) Quercetin.
ProTox-II server estimates the toxicity of a chemical compound and classifies the compound into a toxicity class ranging from 1 to 6. The server classifies the compound according to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). According to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS), since Epigallocatechin gallate had predicted toxicity class was of 4, it would be harmful if swallowed. With the predicted toxicity class of 5, Daidzein might be harmful if swallowed. And Quercetin, with its predicted toxicity class was 3, it was predicted to be toxic if swallowed [104,140].

The Cytochrome P450 (Cyp450) is a superfamily of enzymes that comprises of 57 isoforms of P450 enzymes. These enzymes are heme-containing enzymes. They catalyze the phase-I metabolism of almost 90% of the marketed drugs and convert the lipophilic drugs to more polar compounds. Among the 57 isoforms, 9 most prevalent isoforms are CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6, 2E1, and 3A4 [141,142]. All three best-selected ligands showed multiple SOMs for these nine isoforms of P450, which indicates that they might be metabolized well by the body.

Frontier orbitals study or DFT calculation is an essential method of determining the pharmacological properties of various small molecules. HOMO and LUMO help to study and understand the chemical reactivity and kinetic stability of small molecules. The term ‘HOMO’ directs to the regions on a small molecule which may receive electrons during a complex formation and the term ‘LUMO’ indicates the regions on a small molecule that may receive electrons from the electron donor(s). The difference in HOMO and LUMO energy is known as gap energy that corresponds to the electronic excitation energy. The compound that has the greater orbital gap energy, tends to be energetically unfavorable to undergo a chemical reaction and vice versa [107,143–146]. All of the ligands were reported to have significant energy gap indicating their possibility to undergo a chemical reaction (Table 9 and Figure 6).

Finally, all the best-performed ligands were analyzed in different post-screening study and they are predicted to perform sound. Overall, this study recommends Epigallocatechin gallate, Daidzein, and Quercetin as the best inhibitors of CDK-2, Human topoisomerase IIa, and VEGFR-2, respectively among all selected ligands which could be potential natural plant-derived compounds to treat cancer. However, other compounds could also be investigated as they were also showed convincing docking scores (Table 2). Further in vivo and in vitro experiments might be required to strengthen the findings of this study.

5. Conclusion

In the experiment, total thirty anti-cancer agents from plants were selected to analyze against three enzymes, CDK-2, Human topoisomerase IIa, and VEGFR-2, of three different pathways that lead to cancer development. Ten ligands were studied for each of the enzyme group using different approaches of the computer-aided drug designing. Upon continuous computational experimentation, Epigallocatechin gallate, Daidzein, and Quercetin were predicted to be the best inhibitors of CDK-2, Human topoisomerase IIa, and VEGFR-2, respectively. Then their drug potentiality was checked in different post-screening studies where they were also predicted to show quite similar and sound performances. However, the authors suggest more wet lab based in vivo and in vitro researches to be performed on these best three agents as well as the other remaining agents to finally confirm their potentiality, safety, and efficacy.

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