ORIGINAL ARTICLE

Computational Screening of Anti-Cancer Phytochemicals: Molecular Docking Simulations and Drug Designing

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ABSTRACT

Background: Cancers are caused by uncontrolled proliferations of malignant cells due to defective apoptosis mechanism therefore, most attractive drug target discovery strategy is to find ligands which have the ability to activate or regulate the apoptotic machinery.
Aim: To scrutinized anticancer attractive drug target ligands from *Arnebia hispidissima, Terminalia arjuna, Digera arvensis forsk* and *Caesalpinia crista*.
Methodology: *In silico* molecular modeling simulations were performed by interactions between active site of receptor residues and potential phytochemical ligand with least energy values and most efficient interactions.
Results: The most effective medicinal plant with significant number of phytochemicals with virtual potential anticancer phytochemical remain *Arnebia hispidissima.* Moreover, the potential phytochemicals from *Terminalia arjuna* possess significant binding affinity including are ursolic acid-2xyj, ursolic acid-5c3h and Ellagic acid-2xyj respectively. Moreover Bcl-2 regulator protein showed maximum amino acid interactions by all the phytochemicals.
Conclusion: This advanced computational drug designing modeling approach therefore, identifies the potential leads against over expressed tumours.
Keywords: Arnebia hispidissima, Terminalia arjuna, Digera arvensis forsk, Caesalpinia crista, antitumor, molecular modeling

INTRODUCTION

Chemotherapeutic agents act mechanistically either by cytostatic or cytotoxic to target site. Some of the most promising results shown by them are in combination therapy with other anticancer drugs[1]. The Molecular docking algorithms are concerned with the evaluation, generation and interpretation of possible intermolecular structural complexes. Most docking algorithms are able to generate a large number of possible structures in terms of docking score and each structure can be identified for its drugability[2]. The two main information to be obtained from a molecular docking simulation are the correct conformation of a ligand-receptor complex and a binding affinity prediction, expressed as docking energy 'Ed'. The docking energy represents an approximation of the binding free energy variation, ΔG binding ≈ Ed, relevant to the complex-formation equilibrium from the free receptor (R) and the free ligand (L)[3].

A protein modeling plays vital role, after identification and validation targeted proteins and their role in cancer enhancement five different proteins and their 3D crystal structures were retrieved. The detailed analysis of the protein structure was performed by PyMol whereas active site of protein was specified by the aid of Auto Doc Tools. Protein preparation was performed manually by a series of steps optimization and addition of hydrogen atoms, removal of steric clashes metal ionization or water molecules where requires before molecular docking .There is large number of medicinal plants used as traditional medicine or folk medicine system of Pakistan, local plants was prepared and scrutinized by *in silico* molecular docking[4], keeping in view following facts: 1. Plants with greater efficacy as subscribed by local healers 2. Plants have available pharmacological and biological evaluation data. 3. Availability and collection from original source 4. Taxonomic characterization of selected plants is available. In this study, modern *in silico* drug designing and development approaches were utilized to develop novel effective drug range with target specificity.

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MATERIALS AND METHODS

Ligand retrieval: To design *in silico* drug through molecular simulations, medicinal plants were selected from Pakistan based indigenous knowledge. The validated information and chemical structure of the available databases provide very concise 3D information's about the selected plants and their phytochemicals. All 3D structures were downloading in XML, SDF and MOL format before converted to the PDB format. The Online Molecular Format Converter Open Babel (http://openbabel.org/wiki) was utilized to convert 3D structures to PDF for performing molecular modeling. Phytochemicals were retrieved from Pub chem, drug bank, Zinc data base a library of 112 phytocompounds was generated and optimization by ligand energy minimization was carried out.
Protein retrieval and preparation: Similarly the 3D structures of (a) Epidermal growth factor Protein PDB ID;

1M17 (b) Crystal structure of Bcl-xL PDB ID; 2YXJ and (c) Apoptosis Proteins PDB ID; 5C3H with 2.3 A° was retrieved from(http://www.rcsb.org) Protein Data Bank (PDB). PyMOL software was used to visualize protein and graphical representation of protein generated alteration in protein residues structures and colours was made accordingly. The prebond ligand and water molecules were removed, protein structure was refined with addition of H-atoms to the appropriate residues leading to minimization of energy.

Molecular Docking: Autodock Vina 4.2 a virtual molecular docking program, was used to perform Molecular docking ligands were maintained in library. Meanwhile, the active site along with binding pocket of protein was successfully lead to implementation of docking algorithm from PDB Sum. The 3D conformations between ligand-protein receptor resulted in automatic generation of nine best docking possess in the form of solution file. By conformations for each hit, scores binding affinities were appraised minimum binding energies and 3D transformation were considered most valuable.

RESULTS

The phytochemicals (ligands) are conveniently available for researchers to perform structure-based virtual screening of drugs from selected four medicinal plants (Table 1). Eventually, energy minimization poses were restored due to glide scoring function which has standard unit kilocalorie per mole (kcal/mol). Set of selected proteins included were epidermal growth factor protein (PDB ID; 1M17), crystal structure of Bcl-xl (PDB ID; 2YXJ) and apoptosis proteins (PDB ID; 5C3H) (Figure 1). The order medicinal plants with highest number of potential ligands *Arnebia hispidissima, Terminalia arjuna, Digera arvensis forsk* and *Caesalpinia crista* respectively (Figure 2). Where these highest scoring phytocompounds includes, *Arnebia hispidissima* (I-XII) (a) Heliotrine (5643), (b) Dihydrocapsaicin (65430), (c) Cinchonain1 (11629),(d) 2,4-Dimethoxybenzylamine (119693), (e) L-gamma-Glutamyl-S-Benzyl-N-[(S)-Carboxy (Phenyl) methyl]-L-Cysteinamide (444051), (f) (7R,8S,9R,10R)-10-amino-7,8,9,10-tetrahydrobenzo [a]pyrene-7,8,9-trio (444395), (g) Luteolin (4444102), (h)N'-[2-(3-methylphenoxy)acetyl]-2-(2-nitrophenoxy) propanehydrazide (4944192), (i)(1-[3-[(2-Pyridin-3-ylquinazolin-4-yl)amino]propyl]azepan-2-one)(50645939),(j)11-dimethyl-11azatricyclo[7.4.1.02,7] tetradeca-2(7),3,5-trien-4-ol (3246),(k) Cyanidin (128861), (l) Shikonin (479503),*Caesalpinia crista* (XIII-XIV) (a)1-(4-methylphenyl)-2-(propan-2-ylamino) ethanol (192962), (b) Neocaesalpin (7335199), *Digera arvensis forsk* (XV-XXII) (a) Multezin (6015), (b) Alphaprodin (6471), (c) Alpha-Tetralone; 1-Tetralone (10724), (d) beta-sitosterol (22228), (e) 3-Methyl-10-(substituted-phenyl) flavins (222841),(f)1-Bromo-2,4-Dimethyl-3,5-Dinitrobenzene (228079),(g) 1,3-Thiazole-2-Carboximidoyl Cyanide (4444352), (h) laurendecumallene (44444962), *Terminalia arjuna* (XXII-XXVIII) (a) Chlorovinyl Methyl Ketone (12693), (b) ursolic acid (58472), (c) Neocuproine (65237), (d) Benzilonium (66248),(e) Punicalagin (44584733).

However the plant with highest scoring ligands with crystal structure of selected proteins was *Terminalia arjuna* with promssing 3D ligand structures and protein docking complex for ursolic acid-2xyj, ursolic acid-5c3h and Ellagic acid-2xyj (Figure 3). The binding affinity and docking score for highest scoring photochemical reveals the potential anticancer effect. As ursolic acid-2xyj (-13.6kj/mol), ursolic acid-5c3h (-11.8 kj/mol) and Ellagic acid-2xyj (-9.5 kj/mol) respectively. The enhancement of potency with differentprotein receptor 2xyj, 5c3h for same ursolic acid is due to the interacting residues as given in Table 2.

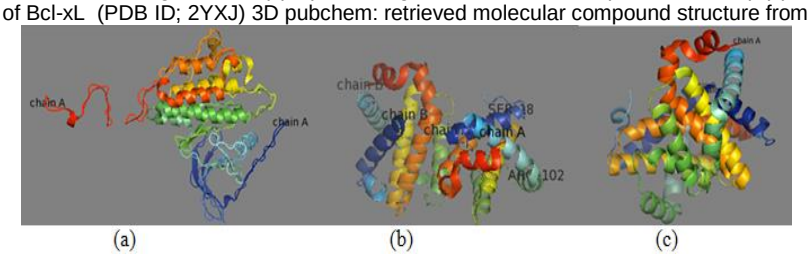
Table 1: Traditional anticancer medicinal plants selected for molecular docking analysis

Botanical Name	Family	Common Name	Traditional use	Reference
Arnebia hispidissima	Boraginaceae	Arabian Primrose	ulcers, antioxidant	10
Caesalpinia crista L.	Fabaceae	Cat's claws,Gray nicker	Antimalarial, tumerogenic medication	11
Digera arvensis Frosk	Amaranthaceae	Drumstick leaves	Antiviral tumerogenic, antibacterial	12
Terminalia arjuana	Combretaceae	Arjan	Leucoderma	(Amalraj and Gopi, 2017

Table 2: Terminalia arjuna binding affinites with target residues

IUPAC names	Mol. Formula	PDB ID	Affinity(kcal/mol)	Residues Interact via H-bonding	Residues in contact To ligand
Punicalagin	$C_{48}H_{28}O_{30}$	2xyj	-.8.1	Phe271, Leu315	Ala344, Leu351, Gln366 Asn391
Punicalagin	$C_{48}H_{28}O_{30}$	1m17	-7.2	Agr258, Val267	Thr261,Ala281,Phe 391, Pro396
Ursolic acid	$C_{30}H_{48}O_3$	5c3h	-11.8	Leu234, Cys256	Ala 186, Asp189,trp11,Gly196
Ursolic acid	$C_{30}H_{48}O_3$	2xyj	-13.6	Cys261, Asn286	Phe120, Leu119,Val136,Phe141
Neocuproine	$C_{14}H_{12}N_2$	2xyj	-7.6	Leu782, Ala793	Asp857,Thr867,Pro877,Ala892
Ellagic acid	$C_{14}H_6O_8$	2xyj	-.9.5	Met 721, Lys735	Leu838,Val853,Gln869,Ile889

Figure 1: 3D structures of Molecular Docking Proteins (a) Epidermal growth factor Protein (PDB ID; 1M17) (b) Apoptosis Proteins (PDB ID; 5C3H) (c) Crystal structure of Bcl-xL (PDB ID; 2YXJ) 3D pubchem: retrieved molecular compound structure from selected medicinal plants



(a) (b) (c)

Figure 2: 3D ligand structuresa, chemical name (pubchem ID) *Arnebia hispidissima* (I-XII), *Caesalpinia crista (*XIII-XIV), *Digera arvensis forsk* (XV-XXII) and *Terminalia arjuna* (XXII-XXVIII)

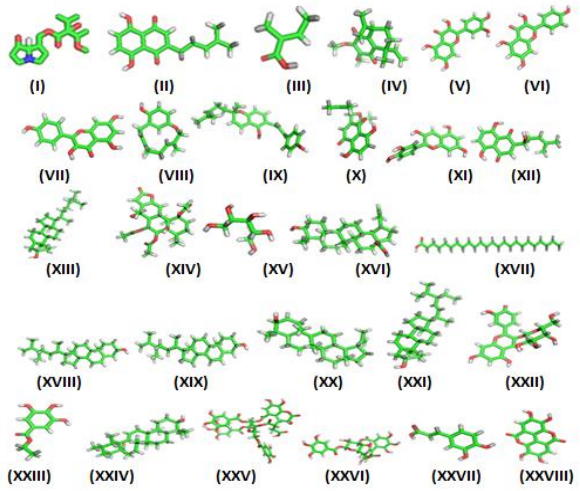
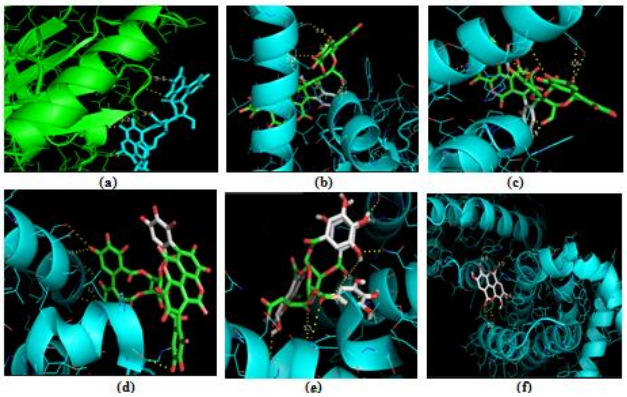


Figure 3: Terminalia arjuna 3D ligand structures and Protein docking complex (a) Punicalagin-2xyj (b) ursolic acid-5c3h (c) Punicalagin-1m17 (d) Neocuproine-2xyj (e) Ellagic acid-2xyj (g) (f) ursolic acid-2xyj



DISCUSSION

The selected medicinal plant and their secondary metabolites, flavonoids, flavonols, saponins, terpenoids, alkaloids etc have significant role either in inhibiting or suppressing onco–proteins available in the literature were obtained from different software free scientific databases[5]. In this research numerous computational molecular models were employed for specific information like the binding site, geometry, physiochemical properties and electrostatic potential. These factors aid to identify an optimal binding site for potential desired drug molecule by virtual high throughput drug screening[6]. To avoid failure of drug at latter stages, pharmacokinetics and pharmacodynamics properties of lead were estimated by Lipinski rule of five was to limit the rejection and enhance the efficacy thus 18 compounds were rejected through this filter and compounded repeatedly present in several plants were proceeded one. The high throughput virtual screenings of total one hundred twelve ligands were selected from medicinal plants. Top hits scoring phytochemicals were selected the scoring function provides information about in silico efficiency highest binding affinities, polar bonds and interactions with active residues[7]. Moreover, crystal structure of B cell lymphoma a member of crystal structure of Bcl-xl (PDB ID; 2YXJ) of Bcl-2 regulator protein family which regulates the cell death and apoptosis protein showed maximum amino acid interactions by all the compounds in study. On the basis of hierarchical filters, the ligand-protein interaction function was evaluated the glide program supports necessary and favorable interactions this filter test made ligand to be activated for protein spatial fit and assess the interactions by grid method[8]. During the interactive ursolic acid performed with maximum anticancer inhibition as evaluated in previous studies[9].

Present study shows, the lead compounds selected binds efficiently to target molecule poses lowest energy and expected to be a better drug candidate *in vitro*. The non-covalent, hydrogen bonding predicts the robust binding interactions between ligand and protein residues almost all compound showed 2-4 hydrogen bond in different more conformations. The present study provides the base for *in vitro*, animal model and clinical trials in the form of target specified direct acting anticancer potential drug. Furthermore, in this *in silico* computational simulations provides high throughput screening of hundred and twelve drug candidates with than three hundred resulted suggested simulations provides detailed information within minimum possible time and cost. However, further wet lab experiments are recommended for mechanistic aspects and drug development.

CONCLUSION

Current computer aided simulation analysis reveals binding affinity and the highest number of effective anticancer phytochemicals is *Arnebia hispidissima*. Interestingly, the plant with three top scoring reporter inhibiting phytocompounds in throughput virtual screening belong to the same plant *Terminalia arjuna*. These phytochemical with specific binding potential to successfully block proteins ursolic acid-2xyj, ursolic acid-5c3h and Ellagic acid-2xyj which can provide a basis to develop a promising antitumor drug after modest structural modifications as per *in vitro*, animal model and clinical trials.

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Declaration of Interest: The authors declare no conflict of interest.

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