Reverse Pharmacognostical Evaluation of Cissus Quandrangularis

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Abstract

This study was aimed on the reverse pharmacogonocy evaluation of Cissus Qquandrangularis. Study targeted on the chemical compounds available on C. quandrangularis responsible for antidiabetic activity. Molegro Virtual Docker (MVD) docking software was used to identify putative binding biological targets for binding of C. quadrangularis derived biologically active compounds. Among the 200 screened proteins five targets were retained: Peroxisome proliferator-activated receptor alpha (4ema), Glucokinase (5brh), Glycogen Phosphorylase (1a8i), Dipeptidyl Peptidase IV (1orv) and Fructose-1, 6-bisphosphatase (1umg). Binding test was realized for these five protein candidates as well as one reference. The predictions made by MVD were consistent with the experimental results, significance that these five targets can be modified by an extract containing this compound in an appropriate concentration. These results exhibit that reverse pharmacognosy and its inverse docking component is an influential tool to identify biological properties for natural molecules and hence for plants containing these compounds. In the case of Cissus Quandragularis some chemical constituents responsible for the antidiabetic activity and was proved to be very effective, and some chemical compounds are identified.

Key Words: Reverse, natural, results, reference

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Introduction

The need for new drugs in certain therapeutic areas e.g. antibiotics and oncology are obvious. Natural substances are still good sources for finding new active molecules [1]. Pharmacognostic field studies have led to many successes in identifying molecules of interest from living organisms such as plants. The identification process goes through several steps (i) organism selection- ethnopharmacology is helpful in identifying particular organisms used in traditional medicine (ii) iterative activity-guided fractionation and finally (iii) active molecule(s) purification, identification and characterization [2, 3]. Methods to exploit traditional knowledge based on computational techniques have demonstrated the utility of such strategies [4, 6]. New approaches inspired by pharmacognosy have been introduced such as “reverse pharmacology” (RPL) or “reverse pharmacognosy” (RPn) [7, 8]. The aim of reverse pharmacognosy is to find new biological targets for natural compounds by virtual or real screening and identify natural resources that contain the active molecules. RPn is similar to “reverse pharmacology” as small molecules are used as probes to evaluate their effects on a biological system, but differs from reverse pharmacology by its final goal. RPn allies chemoinformatics tools and traditional knowledge in the search for the plants aimed at the development of botanicals, pharmaceuticals and cosmetics. In the first step, the biological properties of a molecule are screened in-slico and/or in vitro, then in a second step, it is possible to retrieve the plants containing the active compounds thanks to a plant/molecule relational database. Thus reverse pharmacognosy is complementary to pharmacognosy.

The starting material for pharmacognosy is raw plants selected by allowing for their traditional uses or by biodiversity. Extracts are prepared from the plants and screened on biological assays to find active ones and the path shown in fig. 1.

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The active compounds will be measured by an iterative bio-guided fractionation. So pharmacognosy starts with plants and ended with molecules. Reverse pharmacognosy may be undertaken with or without a virtual section. Molecules are chosen by chemical diversity. They are screened on several biological assays. Molegro virtual docker can be introduced prior to the biological screenings.

Two key components are necessary for implementing reverse pharmacognosy: (i) a virtual screening tool such as Selnergy or a real screening platform; (ii) a database linking plants and molecules. Molegro virtual docker is docking software and a 3-dimensional (3D) protein target database, and helps to targets that can interact with a molecule [9]. Some authors used Dock as the docking tool. This software unluckily suffers from a lack of speed and precision so is not suitable for a systematic screening of a huge target database. Similar works have been published that used other docking tool [10]. Besides, we brought particular attention to the quality of the protein models we have incorporated into our target database by implementing a selection method (see Materials and Methods). A visual analysis is finally applied to the best hits from Molegro to discard false positive.

Here we report the study of coumarin derivative characterized by an epoxide group. The purpose is to find an application for this product and as a result an application for its source.

Antidiabetic activity

Plant extracts are attractive sources of new drugs and have been shown to produce promising results in the treatment of many diseases [11,12]. On the basis of above evidence it is possible that some phytoconstituents like Flavonoids, Phytosterol, Tannins, and Triterpene present in the Plant extracts (ethanol extract) are responsible for the observed antidiabetic activity. There are so many chemical compounds present in cissus quadrangularis for antidiabetic activity but according to the research articles flavonoids are more responsible for activity [13" 16]. They contain polyhydroxy group with aromatic ring which shows maximum interaction with the enzyme. For antidiabetic activity five enzymes was selected such as Peroxisome proliferator activated receptor alpha (ppar), glucokinase, glycogen phosphorylase (GPa), dipeptideyl peptidase (dpp4) and fructose 1-, 6 diphosphate (F16DP). These enzymes are the main target for antidiabetic activity.

Various parts of Reverse Pharmacognosy

Structural database for natural compounds

Natural compounds that appear in the published literature and compounds found in commercial databases forms the structural database also called Virtual Chemical Database (VCDB). The sources of these compounds are accessible, and frequently the method applied for their extraction is also defined. CONCORD is a VCDB contains more than 100,000 natural compounds, along with their 3D coordinates. Chemical diversity of the compound is the final criterion for the selection of compounds for virtual screening [4].

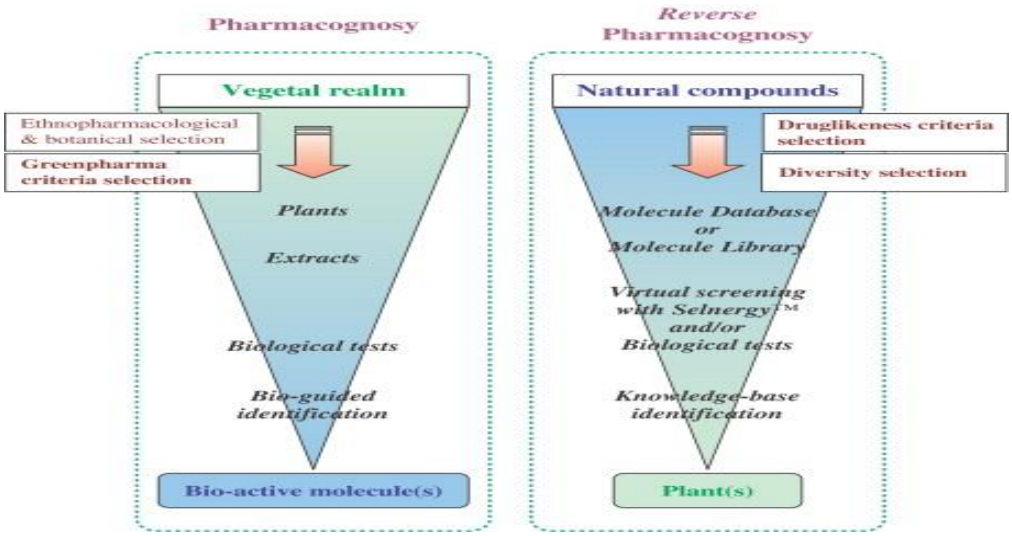


Fig 1: Reverse Pharmacogonocy Path

Target database

The target database is composed of 3D protein structures, determined by X-ray crystallography or by homology modeling. The majority of the structures are from humans, although it also contains proteins from other sources (eg. viruses, bacteria). Protein/ligand pair interaction energy is obtained and involves recovery of active ligands from a set of 100 non active compounds method is explained in detailed by Greenpharma [17].

Virtual screening tools

The basic goal of virtual screening is the decrease of the huge virtual chemical space of small organic molecules, to synthesize and screen against a specific target protein, to a manageable number of compounds that exhibit the highest chance to lead to a drug candidate [18]. There are two methods for virtual screening : screening based on ligand properties, i.e physicochemical properties (one-dimensional data), fragmental explanation (two-dimensional data) and pharmacophores (3D data) which are techniques of quantitative structure-activity relationship (QSAR)and screening based on target properties [19] , which needs knowledge of the 3D structure of the target and the ligand which are techniques of de novo design and docking, which involves producing new ligands or adjusting ligands in the active site of the target, respectively [20,21].

Ethnopharmacological database (ETPHDB)

In order to develop botanical data, natural chemical structures, biological testing of extracts and compounds, ETPHDB has been developed. Family, genus, species, common names and synonyms of the plants are included in this database [22]. The database accelerates the discovery of bioactive elements e.g. anti-inflammatory compounds. The ETPHDB contains botanical information on plants and their traditional uses, and phytochemistry data associated with biological activity of plants, database allows being a link between plants, molecule and activity [23, 24, 25].

Experimental Section

Molecular Modeling

Molegro Virtual Docker

Molegro Virtual Docker is based on interactive optimization techniques inspired by Darwinian evolution theory (evolutionary algorithms, EA). A population of individuals (candidate solutions) is exposed to competitive selection that weeds out poor solutions. Recombination and mutation are used to generate new solutions. It consists in an objective library from crystallography (structures retrieved from Protein Data Bank http://www.rcsb.org/pdb/) or from homology modeling [26]. A protein structure is considered "validated" if it can retrieve active ligands from a set of randomly selected molecules. In other words, the active molecules should be best scored. Only validated structures are added into our target library. The scoring of ligand placements in proteins is done by R rank Score from the Tripos package. Lipinski's properties such as molecular weight, log P and number of hydrogen bond donors and acceptors were taken from the PubChem database for Cissus quadrangularis derived plant compounds [27].

Molecule drawing

All compounds was sketched in ChemDrawUltra 2D 8.0 and three dimensional structure was calculated with Chem3D Ultra 8.0 and minimized with MM2, MOPAC method.

Docking of compounds

The three dimensional structure of the all five enzyme Peroxisome proliferator-activated receptor alpha (4ema), Glucokinase (5brh), Glycogen phosphorylase (1a8i), Dipeptidyl Peptidase IV (1orv) and fructose-1, 6-bisphosphatase (1umg) was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb). The RCSB PDB is a source for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. It provides simple and advanced searches based on annotations related to sequence, structure and function. Compounds were considered for flexible in the docking procedure. A rigid core with a maximum of interaction groups (e.g. donors and acceptors of hydrogen bonds) is the first selected, then placed inside the active site of protein. After that, the flexible parts of the molecule are iteratively grown with respect to steric and electroststic constrains of the protein pocket. Dissimilar molecule poses are scored and ranked. The best scored ligand and its protein was selected for further interpretation to its antidiabetic activity.

Hit selection procedure

In the inverse docking post processing, a spatial fitting is taken into account for determining whether a molecule is "correctly" docked into a protein active

site. This is achieved by comparing the distance between the centroid defined in the active site of the studied protein and the centroid defined in the studied ligand (distance centroid to centroid: dC-C). A molecule with a dC-C > 4 Å is considered out of the protein active site and the protein/ligand couple is discarded from further analysis. Hit identification is based on estimated interaction energy of a molecule with a target divided by the number of the molecule atoms (E1). This energy is referred as "normalized interaction energy". This normalized energy is then compared with a reference ligand normalized interaction Energy (ligand known to interact with the protein) referred as E2. E1 should be less or equal to E2 for a solution to be accepted. We used the interaction energy normalized by atom number because huge molecules have the tendency to be better scored by docking and scoring tools, thus biasing the docking.

Results

All the protein structures that fulfill this criterion which are selected. We will focus on the five high scored ligand bind to the proteins. Glycogen phosphorylase is the best scored protein for the antidiabetic activity. It shows maximum interaction with the binding pocket of enzyme. The second protein selected is the Peroxisome proliferator-activated receptor alpha. And the third one the rational binding protein, a fructose-1, 6-bisphosphatase then Glucokinase and Dipeptidyl Peptidase IV. The pair of molecule/protein with a negative E1-E2 is selected and values are listed in table 1 and 2.

Table 1: Molecular dock score of ligand with different enzyme

Ligand (E1)	Enzyme	Mol dock score
Pallidol	Peroxisome proliferator-activated receptor alpha	-165.213
Methyl tetracosanoic acid	Glucokinase	-138.122
Parthenocissin	Glycogen phosphorylase	-189.314
Methyl linoleate	Dipeptidyl Peptidase IV	-117.141
Stigmasterol	fructose-1,6-bisphosphatase	-160.573

Table 2: Molecular dock score of reference ligand with different enzyme

Reference Ligand (E2)	Enzyme	Mol dock score
Glibenclamide	Peroxisome proliferator-activated receptor alpha	-182.79
	Glucokinase	-154.155
	Glycogen phosphorylase	-192.68
	Dipeptidyl Peptidase IV	-120.07
	fructose-1,6-bisphosphatase	-181.19

Table 3: Affinity prediction from Molegro Virtual docker "Code" column contains Protein Data Bank codes for selected proteins

Compound	Code	Protein	E1-E2
Pallidol	4ema	Peroxisome proliferator-activated receptor alpha	-17.578
Methyl tetracosanoic acid	5brh	Glucokinase	-16.033
Parthenocissin	1a8i	Glycogen phosphorylase	-3.375
Methyl linoleate	1orv	Dipeptidyl Peptidase IV	-2.93
Stigmasterol	1umg	fructose-1,6-bisphosphatase	-20.625

Because the number of interaction sites can be high in a protein active site, docking software can sometimes place a ligand in a wrong pose. In order to discard false positive, we have to examine visually the docking results. The active site of glycogen phosphorylase is a deep close pocket surrounding mostly by hydrophobic residues. Parthenocissin is a hydrobhobic molecule, it is docked into the active site and form hydrobhobic intraction with residue of glycogen phosphorylase shown in fig 3. The hydroxy

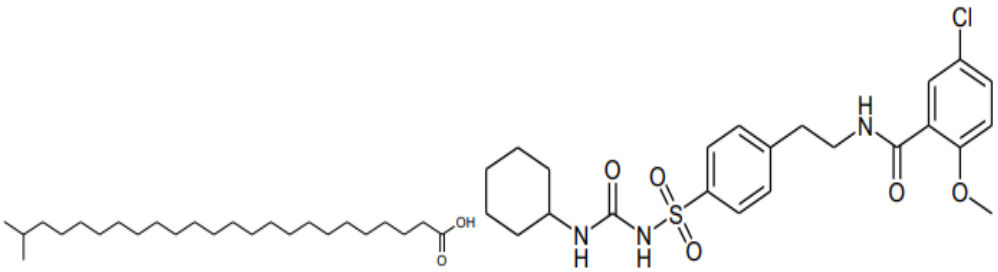
fregments is located in the outer surface of the ligand and shows interaction with ASP283, GLN665, VAL567, TYR90, LYS608 and TYR648. The hydroxyl group of the panthenocissin may form a hydrogen bond with the oxygen and nitrogen group of residue. And then pallidol shows high affinity for peroxisome proliferator-activated receptor alpha to the hydrophopic pocket which is shown in fig. 2 and 3. Pallidol seem to be well docked into ppar-alpha. The polar group of the pallidol is positioned close to seven residue that form hydrogen and/or ionic interaction with the hydroxy group of the ligand a this hydroxy group may form hydrogen bonds with LEU340, GLU343, LEU228, LYS230, LLE326 and SER342. At the vicinity of this polar residue, a hydrophobic cleft can be found in which hydrophobic molecule can be fit.

The third fructose-1, 6-bisphosphatase protein by molegro virtual docker binding protein to the sigmasterol ligand. It shows only two interactions with PRO197 and GLU135. These data consider, to the binding protein appears a less likely binding partner for compound. For that reason affinity of sigmasterol may be questionable. In case of Glucokinase it shows maximum docking score to the methyl tetracosanoic acid and bond with GAL32 and VAL80. The last residue is dipeptidyl peptidase IV give maximum score to the methyl linoleate ligand and share hydrogen bond with ARG358 and PHE357. These results shows best scored ligand with its residue may have affinity for antidiabetic activity and those ligands are responsible for the maximum interaction with the active site. Graphical representation of glibenclamide with other drugs is given blow.

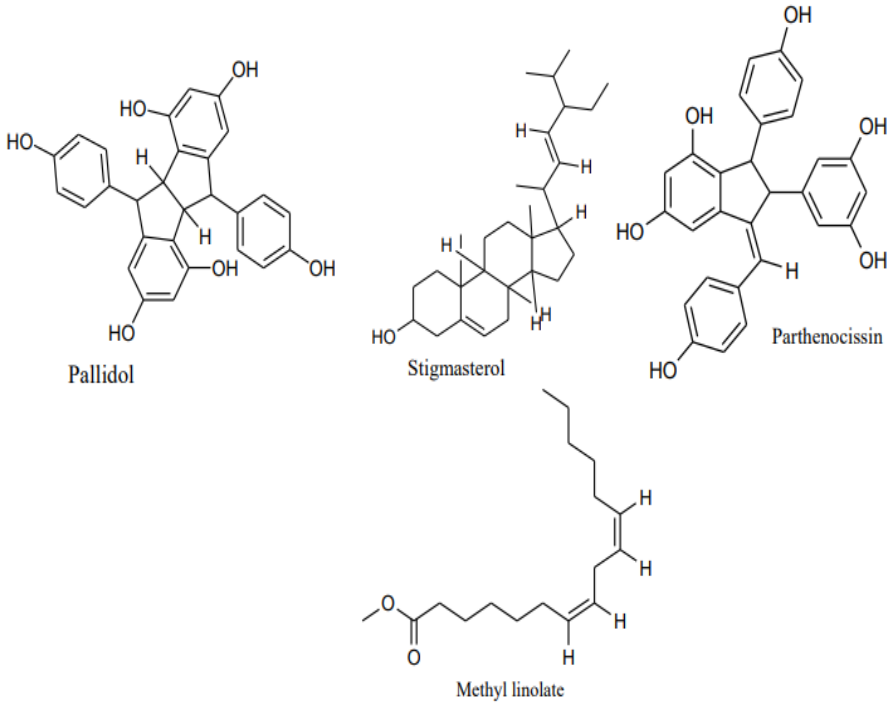
Discussion

RPG is a highly valued asset in efforts to discover new activities among natural compounds and organisms enclosing them. This method is not proposed to replace pharmacognosy research methods but rather arrange for a balancing strategy as well as a new way to study natural compounds and their potential applications. Different tools are required in a RPG process: chemoinformatic implements to handle molecules, inverse screening software with a protein structure database to predict new therapeutic activities, and then a natural compound/source database to position organisms in new actions. Moreover, traditional medicine data help to associate biological activities with traditional uses. These heterogeneous data are sparingly available on the Internet. We studied related databases, all of which are attentive and found that - possessing their own purposes - they are unfortunately not responsive to RPG work. In practice, users would have to “manually” link apparatuses and data, which all too often present incompatibilities with favor to input, processing and output requirements. In order to solve such problems, the GRPG (Greenpharma Reverse Pharmacognosy platform) platform has been developed. It includes asuitable database by participating heterogeneous data with a unique user interface for an “all-in-one” study in search of molecules, organisms, biomolecular targets and biological activities coupled with collections about traditional uses.

Our method can help to find new application for plants based on medicinal concepts of molecules and protein targets and more intensive on indications. It supplements traditional cure which are more attentive on the origin of disease. Behavior in mind that toxicity also constituents a critical issue, we hope, however that our approach will extend access to treatments for populations that cannot afford western medicine drug.



Methyl tetracosanoic acid Glibenclamide



Conclusion

Our study revealed that inverse docking can be of high interest to find applications of compounds, adding value to such new molecules. Molegro Docker can accelerate this process of known target identification through its indexed target library. In the case of cissus quandragularis some chemical constituents responsible for the antidiabetic activity which may be predict by the high scored ligand parthenocissin and the target enzyme glycogen phosphorylase, prediction was in good agreement with experimental data. Moreover, if future biological tests confirm the activity.

Our in silico tool is obviously valuable for decision making in the process of finding new applications for new molecules. One has to bear in mind that simple models are used to compete with biological complexity so that human supervision with experimental validations will still be critical.

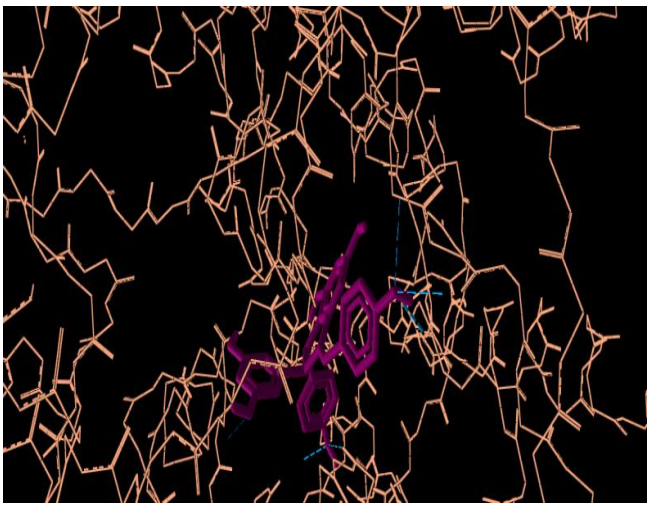


Fig. 2: Docking of parthenocissin with glycogen phosphorylase

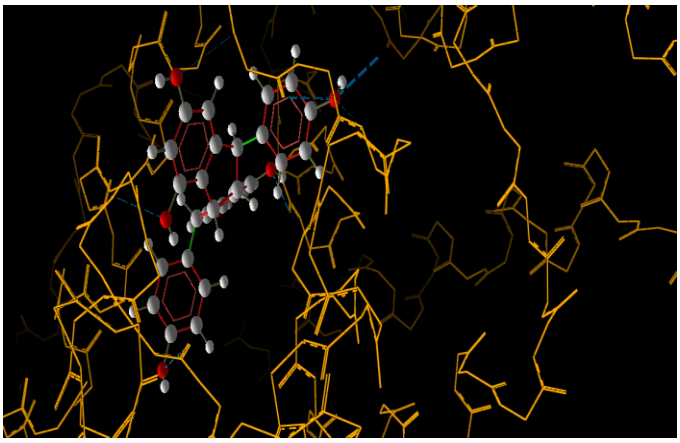


Fig. 3: Docking of pallidol with peroxisome proliferator-activated receptor alpha

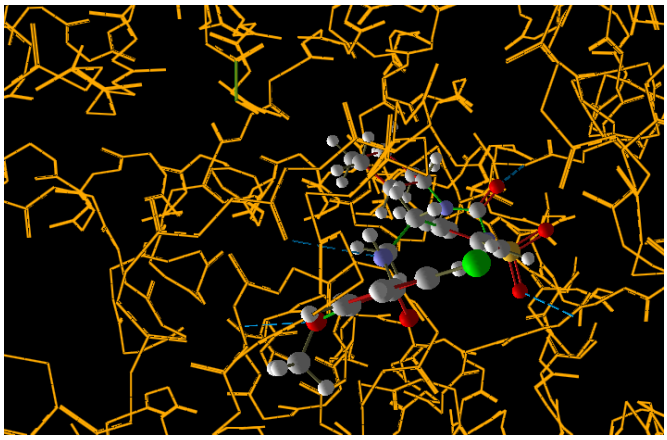


Fig. 4: Docking of glibenclamide with glycogen phosphorylase

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