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REVIEW ARTICLE

Reverse Pharmacognosy: Easy Finding of Lead Molecule

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ABSTRACT
The aim of reverse pharmacognosy is to find new biological targets for natural compounds by virtual or screening and identify natural resources that contain the active molecules. Alternative systems of medicine viz. Ayurveda, Siddha and traditional system of Medicine have become more popular in recent years and have proven their significance in medical sciences. The three main hurdles in the drug development to provide new functional leads are time, money and toxicity which can be reduced by traditional knowledge and experimental database. Reverse pharmacognosy is a new concept of finding new biological targets from structurally similar chemicals and finally finding the natural sources of the biologically active natural compound which contain them. Reverse pharmacognosy utilizes techniques, such as high throughput screening (HTS), virtual screening and a knowledge database containing the traditional uses of plants. The review will focus on the technique used in reverse pharmacognosy along with its importance in natural drug discovery to finding out a lead molecule from the natural source.
Keywords:-*Reverse pharmacognosy, lead molecule, lead optimization, virtual screening.*

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INTRODUCTION

Alternative systems of medicine viz. Ayurveda, Siddha and Chinese Medicine have become more popular in recent years and have proven their significant in medical sciences. Pharmacognosy is the branch of extracting new chemical entities plant and animal origins, but how traditional formulations act is now becoming challenging to scientist and pharmacologist. Combining the strength of knowledge based on traditional systems such as Ayurveda with the dramatic power of combinatorial sciences and high throughput screening will help in the generation of structure activity libraries. [1-4].

Selection of plants

Plants are selected on Ethnopharmacological knowledge when specific therapeutic area is desired for treatment. Using knowledge from different cultures increases the probability and ability to identify effective materials that correspond to the therapeutic area. One important consideration is the availability for sufficient quantities of samples for testing and subsequent development.

Extraction

Extraction can be defined as a process of removal of soluble materials from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. Extraction is generally done by solvents of different polarity.

Biological evaluation

When the estimation of potency of crude drug or its preparation is done by means of its effect on living organisms like bacteria, fungal growth or entire animal, it is known as bioassay. Herbal drugs are screened for their activity by bioassay.

Characterization

Isolation of active compounds from the extract is an important step; it is generally done by chromatographic techniques like TLC, HPTLC and HPLC. Main constituents of the drug are studied with the aid of HPTLC and modern spectroscopic techniques, using HPLC - coupled spectroscopic techniques such as HPLC-UV,HPLC-MS as well as HPLC-NMR.

REVERSE PHARMACOGNOSY

Reverse pharmacognosy is a new concept of finding new biological targets from structurally similar chemicals and finally finding the natural sources of the biologically active natural compound which contain them.

Various parts of Reverse Pharmacognosy:

Structural database for natural compounds

Natural compounds that appear in the published literature and compounds found in commercial database forms the structural database also called Virtual Chemical Database (VCDB). The sources of these compounds are available, and frequently the method applied for their extraction is also described.

Target database

The target database is composed of 3D protein structure, determined by X-ray crystalloagraphy or by homology modeling. The Majority of the structures are form humans, although it also contains proteins from other sources.

Virtual screening tools

The basic goal of virtual screening is the reduction of the enormous 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. There are two methods for virtual screening : screening based on ligand properties, i.e physicochemical properties (one-dimensional data), fragmental description (two-dimensional data) and pharmacophores (3D data) which are techniques of quantitative structure-activity relationship (QSAR) and screening based on target properties, which requires knowledge of the 3D structure of the target and the ligand which are techniques of de novo design and docking, which involves generating new ligands or adjusting ligands in the active site of the target, respectively.[5-9]

Ethnopharmacological database (ETPHDB)

In order to develop botanical data, natural chemical structures, biological testings of extracts and compounds, ETPHDB has been developed. Family, genus, species, common names and synonyms of the plants are included in this database. The database accelerates the discovery of bioactive ingredients 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.

The simple arrows represent the information flow, while the bold dark arrows correspond to the process flow. Data from an ethnopharmacological database (ETPHDB) are utilized for 'knowledge validation' in converting virtual hits to real hits, and to retrieve the sources of compounds.

The experimental validation process consists of internal biological tests and/or data gathered from scientific literature. Real hits or real inactive candidates enable the validation of the target models.

Comparison of Pharmacognosy and Reverse Pharmacognosy

The starting material for pharmacognosy is raw plants selected by considering their traditional uses or by biodiversity. Extracts are made from the plants and screened on biological assays to find active ones. The active compound are characterized by an iterative bio-guided fractionation. So pharmacognosy starts with plants and ends with molecules. Reverse pharmacognosy may be undertaken with or without a virtual component. Molecules are selected by chemical diversity. They are screened on several biological assays. Then with the help of compound source database, plants containing bioactive molecules can be determined. Docking and inverse-docking software can be introduced prior to the biological screenings. This step enables to find ligand/target pairs, thereby reducing drastically the number of compounds to be evaluated and the number of biological assays. The natural sources are then identified by compound source database. Reverse pharmacognosy begins with molecules to get bioactive plants with their biological activity characterized at the molecular level. Traditional Knowledge Digital Library (TKDL): In

order to prevent grant of patents based on Indian Traditional Knowledge, Government of India has undertaken an ambitious project of creating a Traditional Medicine.

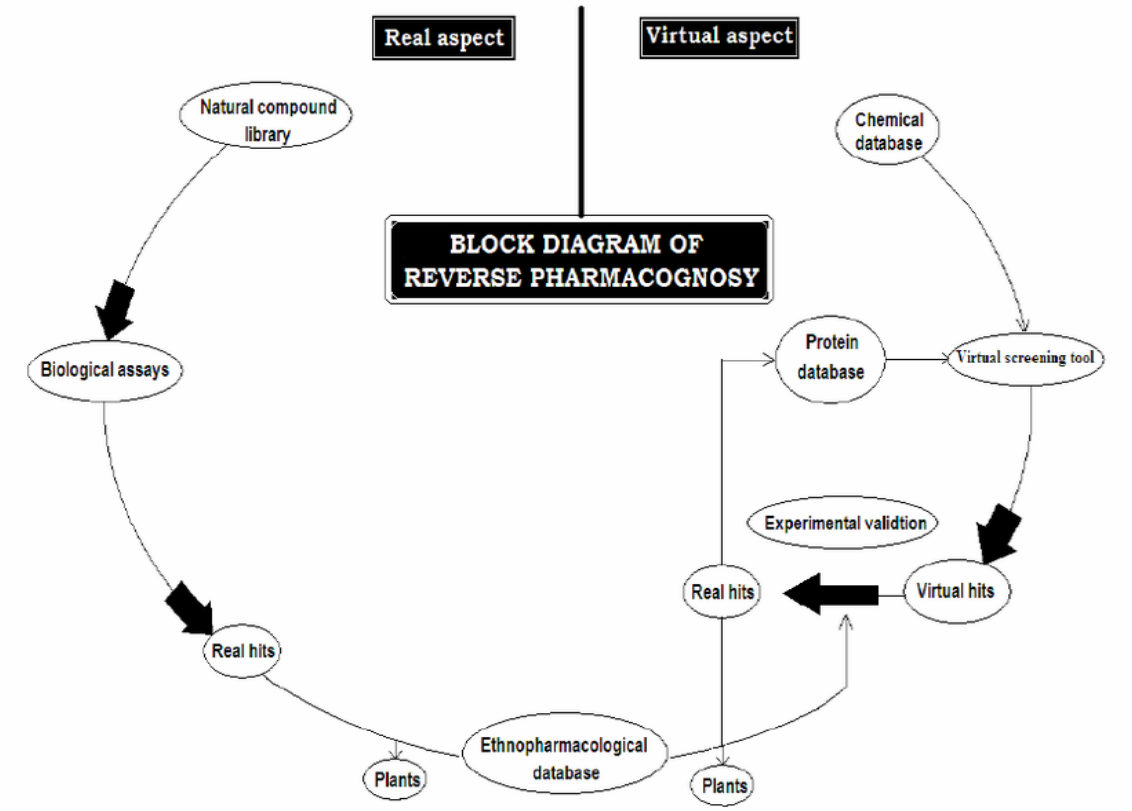

Fig.1: Block representation of reverse pharmacognosy

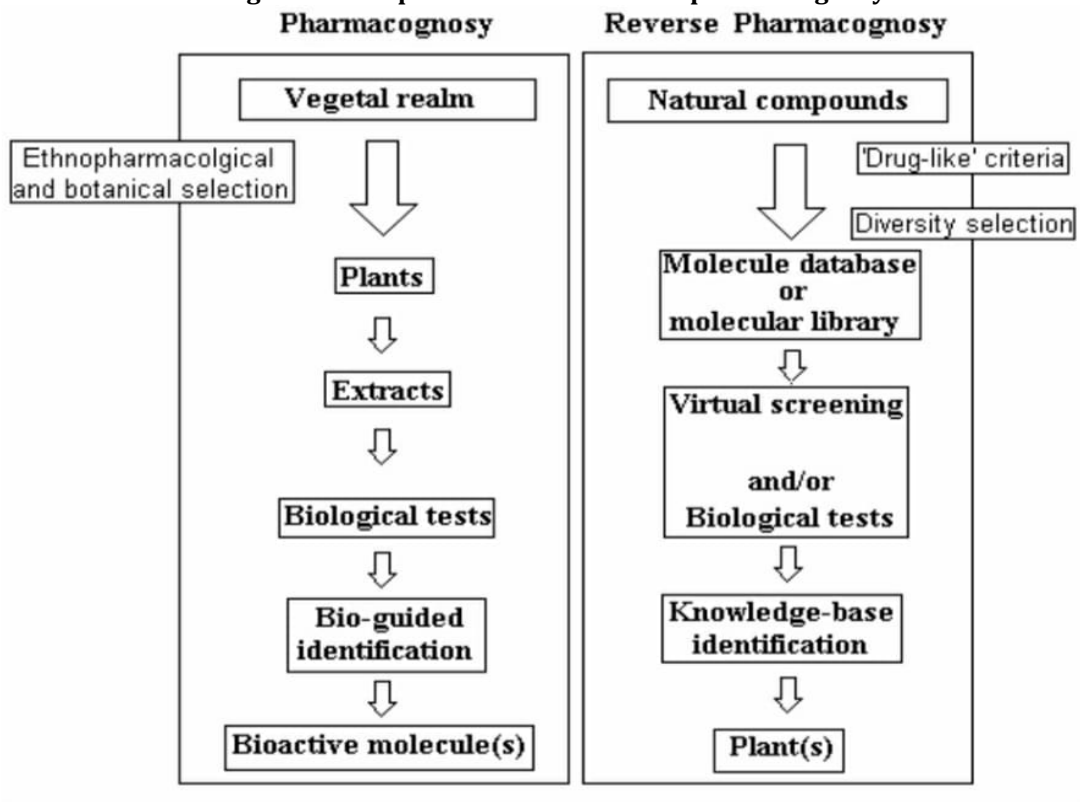

Fig.2: Pharmacognosy and Reverse Pharmacognosy

Knowledge Digital Library. This is a joint venture of the Council of Scientific Research and Central Council for Research in Ayurveda & Siddha. This project is intended to cover about 35,000 formulations available in classical texts of Ayurveda to convert the information in to patent compatible format. The work has been initiated with a cooperative set up of Ayurveda experts, Information Technology experts and Patent examiners. The digital library will include all details in digital format about international patent classification, traditional research classification, Ayurveda terminology, concepts, definitions, classical formulations, doses, disease conditions and references to documents.[10-15]

Target Identification

This step aims to identify a biological drug target. This is typically a receptor, enzyme or ion channel that needs to be manipulated to prevent the development of a disease or alleviate symptoms. Drug usually acts on either cellular or genetic chemicals in the body, known as targets, which are believed to be associated with disease. Scientists use a variety of techniques to identify and isolate a target and learn more about its functions and how these influence disease. Compounds are then identified that have various interactions with drug targets helpful in treatment of a specific disease. Thus, we concentrate our efforts on discovering or even inventing compounds that can alter the disease-causing mechanism, whether a single protein or a complex pathway of proteins, to bring it back into line with normal function. [16-20].

Table 1: Lead compound from natural source and their use

Name of the plant	Lead compound	Activity
Ammit vinsega	Khelluin	Bronchodialator
Berberis species	Berberine	Antidiarrhoeal
Cammelia sinesis	Caffeine	CNS stimulant
Catheranthus roseus	Vincristine	Anti-Cancer
Cassia angistifolia	Sennoside	Laxative
Caphaelis ipecacuanha	Emeine	Anti-amoebic
Claviceps purpurae	Ergometrine,Ergotamine Ergotoxine	Oxytoxic, Vasoconstrictor Vasodialator
Cinchona species	Quinidine, Quinine	Anti-malarial, Anti arrhythmic
Datura species, Hyoscyamus species and Atropa belladinna	Hyoscyamine, Hyoscine Atropa	Para sympatholytic
Dioscorea species	Diosgenin	Anti- inflammatory
Ephedra species	Ephedrine	Sympathomemetic
Erythroxylum coca	Cocaine	Local anaesthetic
Glycorrhiz glabra	Glycyrrhetinic acid	Anti- Inflammatory
Lobelia inflanta	Lobeline	Anti-asthmatic
Papaver somniferum	Morphine, Codeine, Papaverine	Analgesic and sedative
Pilocarpus jaborandi	Pilocarpine	Parasympathomimetic
Plantago ovato	Psyllium mucilage	Laxative
Podophyllum species	Podophyllotoxin	Anti- cancer
Rauwolfia seroentina	Reserpine	Hypotensive, Vasodialator
Solanum species	Solasidine	Harmonal

Target Validation

To select targets most likely to be useful in the development of new treatments for disease, researchers analyze and compare each drug target to others based on their association with a specific disease and their ability to regulate biological and chemical compounds in the body. Tests are conducted to confirm that interactions with the drug target are associated with a desired change in the behavior of diseased cells. Research scientists can then identify compounds that have an effect on the target selected.

Lead Identification

A lead compound or substance is one that believed to have potential to treat disease. Laboratory scientists can compare known substance with new compounds to determine their likelihood of success. Leads are sometimes developed as collections, or libraries, of individual molecules that possess properties needed in a new drug. The most important source of leads is "libraries" of molecule (e.g.) natural product libraries, peptide libraries, carbohydrates libraries, etc. "Virtual libraries" can be created by using combinatorial chemistry. Testing is then done on each of these molecules to confirm its effect on the drug target.

Some of the technologies used in the lead identification are:

1. Virtual screening
2. High throughput docking

Virtual Screening

The dominant technique for the identification of new lead compounds in drug discovery is the physical screening of large libraries of chemicals against a biological target (high throughput screening). Virtual screening is an alternative approach is to computationally screen large libraries of chemicals for compounds that complement targets of known structure, and experimentally test those that are predicted to bind well. It access a large number of possible new ligands which can be purchased and tested. Virtual screening, or *In silico* screening, is a new approach attracting increasing levels of interest in the pharmaceutical industry as a productive and cost-effective technology in the search for novel lead compounds. Although the principles involved the computational analysis of chemical databases to identify compounds appropriate for a given biological receptor-have been pursued for several years in molecular modeling groups, the availability of inexpensive high-performance computing platforms has transformed the process so that increasingly complex and more accurate analyses can be performed on very detailed and relevant basis for prioritizing compounds for biological screening. Virtual screening offers a practical route to discovering new reagents and lead for pharmaceutical research. [21-25]

High throughput docking:

Docking is research technique for predicting whether one molecule will bind to another, usually a protein. Docking is a term used for computational schemes that attempt to find the best matching between two molecules: receptor and a ligand. If the geometry of the pair is complimentary and involves favorable biochemical interactions, the ligand will potentially bind the protein (receptor).

Lead optimization:

Lead optimization compares the properties of various lead compounds to be developed into safe and effective medicines. The candidate drugs with better therapeutic profiles are accessed for quality, taking into account factors such as the ease of synthesis and formulation. After this they are registered as an investigational new drug and submitted for clinical drug.

Testing of the Active Compound (Pre-Clinical Phase)

After optimizing the active compound, testing in the preclinical phase lab and animal testing is used to verify whether it is principally suited for use in the human body. To determine this, the researchers examine among other things how the compound is absorbed by the body, how it is excreted, and how it affects the organs addition, they examine whether and in concentration it has a toxic or effect on the genetic makeup.

Clinical Trials

If the active compound proves successful and also fulfils the legal requirements, it is then directly tested on human beings in three clinical phases:

Phase 1: Compatibility in health test subjects

Phase 2: Determination of optimal doses

Phase 3: Proof of effectiveness.

Approval Process

If the medicine has made its way through all of the preceding phases, the process of getting approval from the authorities begins. The drug cannot be marketed until approval has been obtained.

Drug

Drug is defined as "a chemical substance used in the treatment, cure, prevention, or diagnosis of disease or used to otherwise enhance physical or mental well-being. Drugs may be prescribed for a limited duration, or on a regular basis for chronic disorders.

Target

A drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen. Drugs are used to stop the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Drugs may be designed that bind to the active region and inhibit this key molecule.

Ligand

A ligand is a molecule that is able to bind and form a complex with a biomolecule to serve a biological purpose. It is an effector molecule binding to a site on a target protein, by intermolecular forces such as ionic bonds, hydrogen bonds and Van der Waal forces. The docking (association) is usually reversible (dissociation). Actual irreversible covalent binding between a ligand and its target molecule is rare in biological systems. Ligand binding to receptors alters the chemical conformation, i.e. the three dimensional shape of the receptor protein. The conformational state of a receptor protein determines the functional state of a receptor. The tendency or strength of binding is called affinity. Ligands include substrates, inhibitors, activators, and neurotransmitters.

Fig.3: Drug discovery process

IC50

The **IC50** is a measure of drug effectiveness. It indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process by half. In other words, it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC50). It is commonly used as a measure of antagonist drug potency in pharmacological research. IC50 represents the concentration of a drug that is required for 50% inhibition *in vitro.*

Docking

Docking is the process by which two molecules fit together in 3D space. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Molecular docking may be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. The focus of molecular docking is to computationally stimulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized. [16-20]

Approaches to Molecular Docking

Two approaches are particularly popular within the molecular docking community. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pair wise interaction energies are calculated. Both approaches have significant advantages as well as some limitations.

Shape Complementarily Methods

Geometric matching shape complementary methods describe the protein and ligand as a set of features that make them dockable. These features may include molecular surface complementary surface descriptors. In this case, the receptor's molecular surface is described in terms of its solvent-accessible surface area and the ligand's molecular surface is described in terms of its matching surface description. The complementarity between the two surfaces amounts to the shape matching description that may help finding the complementary pose of docking the target and the ligand molecules. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Yet another approach is to use a Fourier shape descriptor technique described. Whereas the shape complementarity based approaches are typically fast and robust, they cannot usually model the movements or dynamic changes in the ligand/ protein conformations accurately, although recent developments allow these methods to investigate ligand flexibility. Shape complementarity methods can quickly scan through several thousand ligands in a matter of seconds and actually figure out whether they can bind at the proteins active site, and are usually scalable to even protein-protein interactions. They are also much more amenable to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.

Simulation Processes

The simulation of the docking process as such is a much more complicated process. In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein's active site after a certain number of "moves" in its conformational space. The moves incorporate rigid body transformations such as translations and rotations, as well as internal changes to the ligand's structure including torsion angle rotations. Each of these moves in the conformation space of the ligand induces a total energetic cost of the system, and hence after every move the total energy of the system is calculated. The obvious advantage of the method is that it is more amenable to incorporating ligand flexibility into its modeling whereas shape complementarity techniques have to use some ingenious methods to incorporate flexibility in ligands. Another advantage is that the process is physically closer to what happens in reality, when the protein and ligand approach each other after molecular recognition. A clear disadvantage of this technique is that it takes longer time to evaluate the optimal pose of binding since they have to explore a rather large energy landscape. However grid-based techniques as well as fast optimization methods have significantly ameliorated these problems.

The Mechanics of Docking

To perform a docking screen, the first requirement is a structure of the protein of interest. Usually the structure has been determined using a biophysical technique such as x-ray crystallography, or less often, NMR spectroscopy. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components: the search algorithm and the scoring function.[20-25]

CONCLUSION

The concept of research is directed to search for the new chemical entities for the treatment of life threatening diseases. The current review is an attempt made to utilize the principles of reverse pharmacology in conjugation with Ayurveda, siddha and traditional other systems of medicine to develop newer strategies for the drug discovery which will offer newer chemical entities with potential biological activities.

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