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Review Article

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Reverse Pharmacology and Network Pharmacology: Principles and Applications in Herbal Medicine

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Abstract

Drugs that were therapeutic were frequently unintentionally found in medicinal plants during the early days of pharmacology. Cost, time, and toxicity are the three main bottlenecks that can be reduced by the recently developed academic field of reverse pharmacology. Reversing the typical "laboratory-to-clinic" drug discovery pipeline approach to "clinic-to-laboratories" is the idea behind reverse pharmacology, which was influenced by traditional knowledge. Reverse pharmacology aims to optimise the safety, efficacy, and acceptability of natural product leads by studying their mechanisms of action at various levels of biological organisation and by applying pertinent sciences to these findings. New Chemical Entities must go through the drawn-out pre-clinical and clinical research phases of the traditional drug discovery and development process. Conversely, the reverse pharmacology path represents a cyclical approach to drug discovery and development. Drug development from natural products, such as anti-malarial, anti-hypertensive, and anti-Parkinson's medicines, can benefit from the application of reverse pharmacology. The one drug, one target, and one illness method used in drug discovery today is becoming less and less effective. Drug research is quickly embracing the idea of developing multi-target therapeutics to treat complex illnesses. In this context, disease mechanisms are defined by network pharmacology as networks that are best targeted by a combination of treatments that function in concert. Recently, network pharmacology has gained popularity as a means of better understanding the mode of action of herbal remedies. Network pharmacology has several benefits, such as controlling the signalling route through several channels, improving therapeutic efficacy, lowering adverse effects, raising clinical trial success rates, and lowering drug discovery expenses. The field of network pharmacology has its roots in traditional Chinese medicine and has developed alongside it. Static networks and dynamic networks are the two broad groups into which network pharmacology-based techniques fall. The main goals of network pharmacology research are to find genes linked to compounds and diseases, build a network, and then analyse and visualise the network. In order to properly validate the interaction between highly active constituents and their potential targets, further network validation is carried out in the end. Several applications for network pharmacology can be found in drug research, pharmacology, and traditional medicine.

Keywords: Reverse Pharmacology, Network Pharmacology, Scope, Network, Drug Discovery, Database, Applications

1. Introduction

To meet the expectations of "chemistry-naïve doctors," "medicine-naïve chemists" controlled the pharmaceutical industry for many years when it came to new drug research. These days, proteomics, metabolomics, and genomics form the foundation of the prevailing worldview. These new babies on the scene frequently lack knowledge about chemistry as well as medicine! It makes sense why there is such a high attrition rate among drug candidates. It is estimated that the average cost of discovering a new medicine is $1.3 billion. Some of the causes of the current drug discovery catastrophe include the non-specific Organisation for Economic Cooperation and Development (OECD) guidelines for toxicity studies, commercial clinical trials conducted by contract research organisations, and the absence of physician engagement in new

drug development. The lengthy and expensive process of bringing a novel medicine to market scares developing countries away from the field. It is reasonable to question whether a paradigm shift in the drug development process is necessary given the 0.01% market yield from the baseline of 100% New Chemical Entities (NCEs). Reverse pharmacology (RP), a model revolution from traditional medicine (TM), is forming as the model of drug discovery and development has wandered and a crisis is approaching. Although this paradigm shift might not seem all that significant, it provides a way to use the therapeutic knowledge accumulated over millennia to find novel drugs [1]. RP is a transdisciplinary effort that has lately gained popularity. It is a new academic field that has the potential to alleviate three primary bottlenecks that are commonly faced in conventional drug development: cost, time, and toxicity. In terms of drug discovery, RP represents a significant paradigm change. An organised path from clinical experiences, experimental observations, and a data base has been constructed, as opposed to the random pursuit of accidental findings. The whole process of drug discovery, design, and development is revolutionised by the integration of powerful new technologies with traditional knowledge and clinical observations [2,3].

Medicinal plants (MPs) were frequently used in folk remedies in the early days of pharmacology, leading to the accidental discovery of therapeutic medicines. The term "drug serendipity" refers to these pleasurable, unintentional discoveries. There are numerous instances of coincidental bedside observations between MPs and their constituents. Drug research and discovery may be based on these kinds of clinical hits. Technological developments in biotechnology, such as combinatorial and asymmetric synthesis or high-throughput screening, have created new avenues for drug discovery. However, because the drug discovery process has grown incredibly costly, risky, and severely inefficient, the business is facing significant challenges. Big Pharma is quite concerned about two things: a severe lack of innovation and the post-marketing failures of blockbuster pharmaceuticals. Based on information from TM, there has consequently been a notable movement in favour of single- to multi-targeted medicines, particularly for polygenic syndrome. As an alternate source of discovery, strategic options based on ethnopharmacology, TMs, and natural product drug discovery are resurfacing. Botanical products that have been verified by science and standardised by technology can be quickly explored through innovative techniques. By lowering and saving costs, this provides an effective foundation for the development of herbal formulations, speeding up the process of discovering and developing botanical drugs [2,4]. The "one gene, one drug, one disease" model has dominated drug discovery for the past few decades, and the primary goal has been to discover ligands that are incredibly selective while avoiding side effects [5,6]. However, rather than being the result of a single gene mutation or malfunction, complex diseases like cancer, diabetes, etc. are often brought on by the malfunction of an entire regulatory network [7]. As a result, focusing solely on one gene may not be sufficient to diagnose and treat complex illnesses. Novel strategies to attack the disease's underlying biological networks are urgently needed [8,9]. Thus, it is essential to comprehend the molecular mechanisms governing disease prognosis in order to combat complex diseases [10]. Nowadays, a lot of pharmacological agents, especially those used to treat diseases, are made from natural sources. Natural products have long been one of humanity's most abundant sources of powerful resources. In the process of finding new drugs, high-throughput approaches have provided a powerful tool for pharmacological efficacy screening of herbal remedies. Predicting the gene networks that MP active chemicals regulate is a novel approach to providing additional insight into how active substances carry out their therapeutic action [11-15].

Effectiveness is a crisis in drug discovery, largely caused by single-target, ineffectual, and symptom-based techniques rather than mechanistic ones. Drug discovery methods based on one drug, one target, and one illness model are becoming less and less effective [16]. Clinical attrition rates for new medication candidates could approach 30% due to lack of safety and efficacy. Additionally, numerous single-gene knockouts show little change in phenotype, with only 34% of single-gene knockouts resulting in illness or death, according to extensive functional genomics research [17-19]. Although single-target techniques may be effective in treating problems caused by a single gene, they are ineffective in treating complex diseases produced by the interaction of numerous genes. In drug discovery, the idea of creating multi-target therapies to treat complicated illnesses like cancer and diabetes is rapidly gaining attention. Considering this, disease mechanisms are defined by network pharmacology (NP) as networks that are best targeted by a combination of drugs that function well together. It has recently gained popularity to employ NP to gain a deeper understanding of the mode of action of herbal remedies [20]. A recent development in bioscience studies is systems biology, which, instead of concentrating on changing a single molecular component of a biological system, looks at the intricate interconnections within systems as a whole. The corollary of rational drug design of "magic bullets" is replaced by NP, a system biology-based paradigm, by looking for multi-target medicines that function as "magic shotguns" on biological networks. Additionally, a network biology study has shown that disease networks are not significantly affected by the removal of individual nodes. The prevailing notion of single-target drug discovery is being challenged by our growing understanding of the importance of network biological systems [21-23]. Because it combines information science and systematic medicine, NP is becoming a new frontier in medicine discovery. Similar to the early phases of nearly all new technologies, NP's initial notion of drug discovery was hazy and may have involved some exaggeration of its benefits. NP is currently a widely employed strategy in the modern drug development process, and it has started to increase. By building a "protein–compound/disease–gene" network, NP is an integrative in silico method that can be used to uncover the processes behind the TMs' synergistic medicinal effects. In turn, this development has caused the paradigm to change from

"one target, one drug" to "network target, multiple component therapeutics" [15].

Though the scientific basis and workings of Ayurveda, the age-old Indian medicinal system, are still largely unknown, the system makes use of complex combinations of different ingredients and bioactive compounds. When attempting to comprehend the potential effects, indications, and mechanisms of drugs, evidence-based Ayurveda can greatly benefit from the application of NP approaches. In order to create a comprehensive and unique medical system, traditional Chinese medicine (TCM) has developed over thousands of years and accumulated a wealth of clinical competence. Administration of TCM herbal formulations is a noteworthy part of TCM theory's holistic thinking and therapy based on the syndrome (ZHENG) difference. People's curiosity about TCM has grown recently. Still, one of the biggest challenges facing evidence-based TCM comprehends the scientific basis of TCM herbal compositions at the molecular and system levels. It has been possible to identify TCM active ingredients and their biological targets thanks to the recent application of state-of-the-art analytical chemistry and chemical biology technologies to characterise commonly used herbs or herbal mixtures. Research on the molecular makeup of herbal formulas is probably going to help increase TCM's adoption rate globally. In several instances, these endeavours have yielded the discovery of TCM-based drugs by contributing to the identification of important active molecules and combinations of ingredients that work well together. Nevertheless, the combinatorial laws and functions of most herbal formulae in complex diseases remain unclear because TCM herbs and herbal formulas comprise a vast number of constituents, and different molecular transformations are involved in diseases and TCM syndromes. Therefore, combining cutting-edge network science with TCM will offer novel approaches and chances to identify bioactive elements and biomarkers, possibly disclosing mechanisms of action and examining the scientific validity of herbal formulae based on intricate biological systems [24].

As the first step towards "TCM network pharmacology," Li proposed a possible connection between TCM Syndrome and molecular networks in 1999. In 2007, he developed a network-based TCM research method and carried out a network analysis for Hot/Cold herbal formulas and Cold/Hot Syndromes. Subsequently, Li introduced a new concept of "Network target" and changed the TCM research framework to become "Herb network-Biological network-Phenotype network" [25,26]. A few years later, he proposed that the "multi-causal and micro-effective" effects of herbal formulations could govern the disease gene network. Li et al. discovered the network regulation effects of the Cold/Hot syndrome formulas in 2007 after using bioinformatics to create the first biomolecular network of the condition in TCM. The same year, Dundee University pharmacologist Andrew L. Hopkins introduced "Network Pharmacology" (Figure 1). As a result, NP is now a prominent subject in pharmaceutical research. A search of the Web of Science (WOS) and Chinese National Knowledge Infrastructure (CNKI) databases revealed a sharp rise in the quantity of articles published in the field of NP in the last several years. Specifically, it has gained momentum in the last few years and is anticipated to emerge as a promising paradigm for the next wave of drug development. Using NP, Pan Jiahu developed a novel drug discovery model in 2009. Because the core concepts of NP and TCM overlap greatly, it has gained popularity as a research issue in systematic pharmacological research more recently, particularly in the area of studying TCM's pharmacodynamic mechanism. Next, in the same year, Li developed a paradigm for TCM prescription research and TCM evidence called the "phenotypic network-biological network-Chinese medicine network." Two years later, he initially presented the concept of "network targets" and developed a cooperative method for network target-based drug combination prediction. The first international standard for NP, "Guidelines for Evaluation Methods in Network Pharmacology," was created and published in 2021 by Li's team in an effort to standardise the viability of data and boost the reliability of outcomes [27].

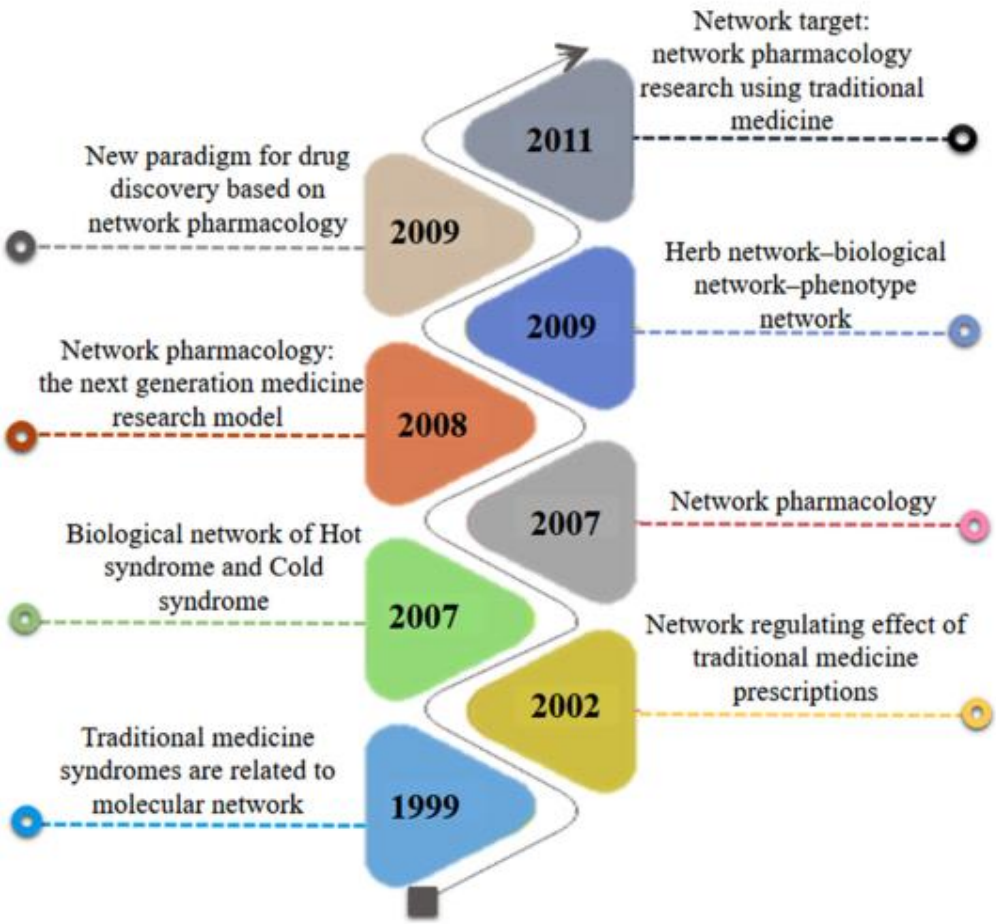

Figure 1: Timeline Diagram Representing the Origin of Np [15]

1.1. What is Reverse Pharmacology?

The science of converting bedside-documented experiential hits into leads through transdisciplinary exploratory studies (*in vitro* and *in vivo*) and then using innovative experimental and clinical research to advance these leads into drug candidates is known as RP [1,28]. Reversing the typical "laboratory-to-clinic" drug discovery pipeline approach to "clinic-to-laboratories" is the focus of RP [2]. The RP route, which emerged from observational therapeutics, is an adjunct to other approaches for the development of natural drugs. Finding structures with unique biodynamic effects may potentially point the way towards developing NCEs for pharmaceuticals [29]. Combining knowledge from traditional or folk medicine with contemporary technology to produce safer and better medicines is a key component of this approach. Examining well-established findings would help identify drug candidates and gain insight into the molecular mechanisms underlying them [2]. The RP method is a sophisticated overhaul of the traditional route for drug development and discovery. The application of RP to traditional therapies makes it easier to find drugs derived from natural ingredients that have been utilised by humans for a long period. In contrast to the conventional (classical) pharmacology approach, which finds new drugs from NCEs, the initiative in the RP path is taken at the bedside. The word "reverse" is explained by the path going from "the bedside to benches" rather than "benches to bedside" (Figure 2). Trans-disciplinary specialists are required by RP, including TM specialists, clinical investigators, basic scientists, clinical pharmacologists, and drug discovery science experts [30].

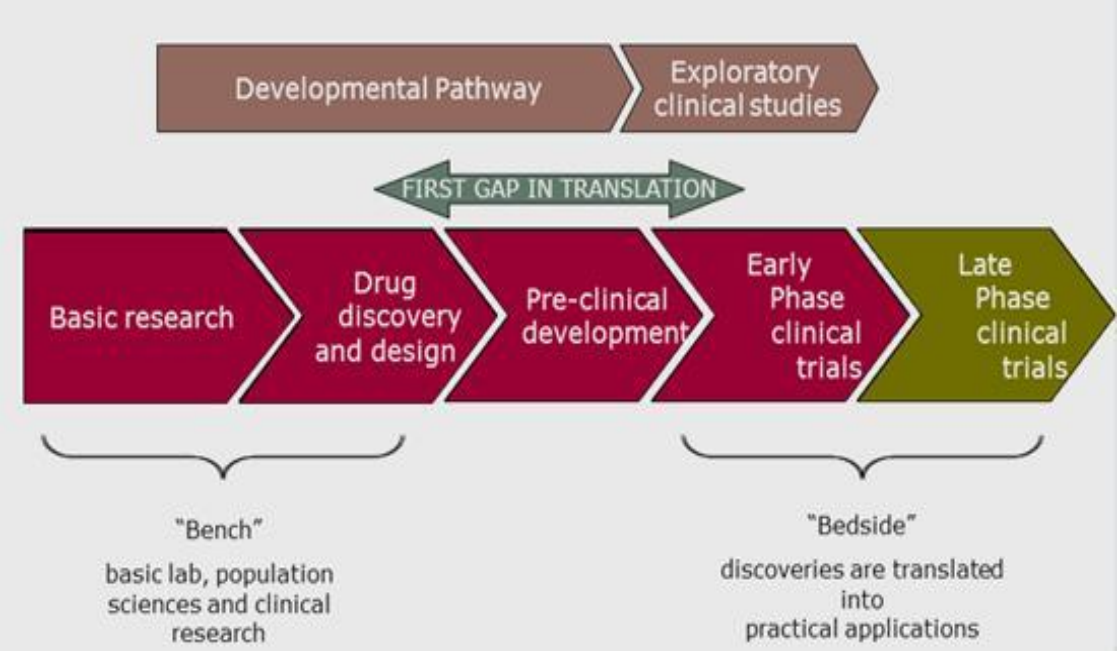

Figure 2: Fast Tract Pharmacology (RP) [31]

1.2. Classical Versus Reverse Pharmacology

Classical (forward) pharmacology and RP are two opposing approaches used in the drug development process [32]. It takes a long time and is ineffective to screen drugs using classical pharmacological approaches (Figure 3). Researchers are increasingly moving away from the approach taken in conventional pharmacological research for novel medicine discovery, using genomes, proteomics, and metabolomics together with modern technology. RP offers numerous benefits. A decade or more is often needed for the classical way to launch a new medicine; however, the reverse pharmacological strategy can complete the process in less than five years and with more efficiency (Table 1) [2].

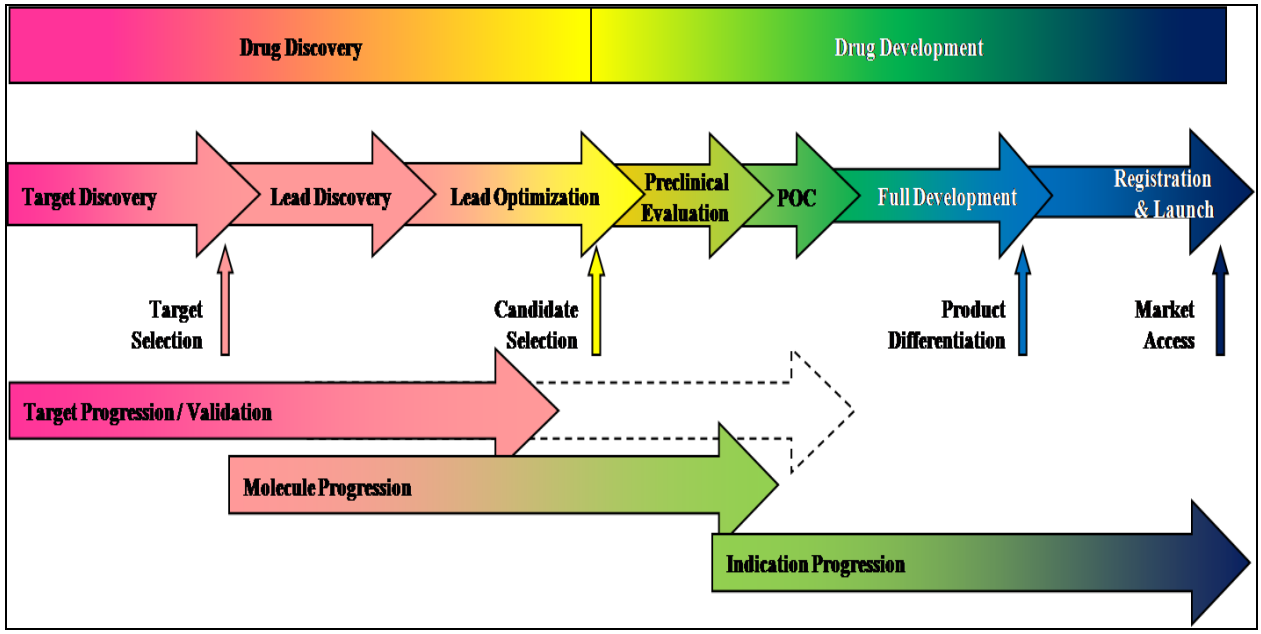

Figure 3: Slow Track Pharmacology (Forward Pharmacology); POC, Proof of Concept [31]

The initial idea in the field of target-based drug discovery (TDD) is that therapeutic benefits will result from changing the activity of a particular protein target that is assumed to modify disease. After that, substances that bind to the target with a high affinity are found by screening chemical libraries of tiny molecules. Afterwards, drug discovery begins using the hits from these screens. The rapid cloning and synthesis of vast amounts of purified proteins made possible by the sequencing of the human genome led to the widespread use of this technique. In current drug discovery, this method is the most popular. Differently than the forward pharmacology, with the RP approach in vivo efficacy of identified active (lead) compounds is usually performed in the final drug discovery stages [32].

Classical Pharmacology	Reverse Pharmacology
Target selection	Clinical Trials (Phase I and II)
Lead compound identification	Mechanistic pre-clinical trials
Molecular target identification	Safety studies
Lead compound optimization	Molecular pharmacological studies
Pre-clinical trials	Large scale clinical trials
Clinical trials	
Expensive, time consuming	Cost effective, time sparing
Drug to market 8-10 years	Drug to market 4-5 years

Table 1: Differences in Pathways of Classical and Reverse Pharmacology [32]

1.3. Scope of Reverse Pharmacology

In order to optimise the safety, effectiveness, and acceptability of the leads derived from natural substances, RP aims to better understand the mechanisms of action at various levels of biological organisation through pertinent sciences. New medicinal chemistry can be developed using the phytoactive components as chemical scaffolds. There are three major domains of RP (Figure 4):

1. The experiential domain covers literature search, ayurvedic pharmacoepidemiology, and well-defined, modest observational therapeutic studies with objective targets of response. This phase includes the meticulous recording of robust clinical observations of the biodynamic effects of formulations used in folk medicine or already-developed drug formulations and their precursors.
2. The exploratory animal studies cover indication-relevant para-clinical studies (in vivo and in vitro studies) for efficacy (target activity) and safety and human pharmacology (tolerability and drug interactions) and phase 2 studies for dose-finding in ambulant patients with defined subsets of the disease.
3. The experimental domain involves well-plannsed experimental and clinical investigations at different levels of biological organisation, extended clinical trials, and sub-acute safety studies in two animal species. RP can be a bridge for translational medicine from the TM to clinical practice. Such an effort would make healthcare delivery locally relevant and may lead to global discoveries in natural products [1,2,28].

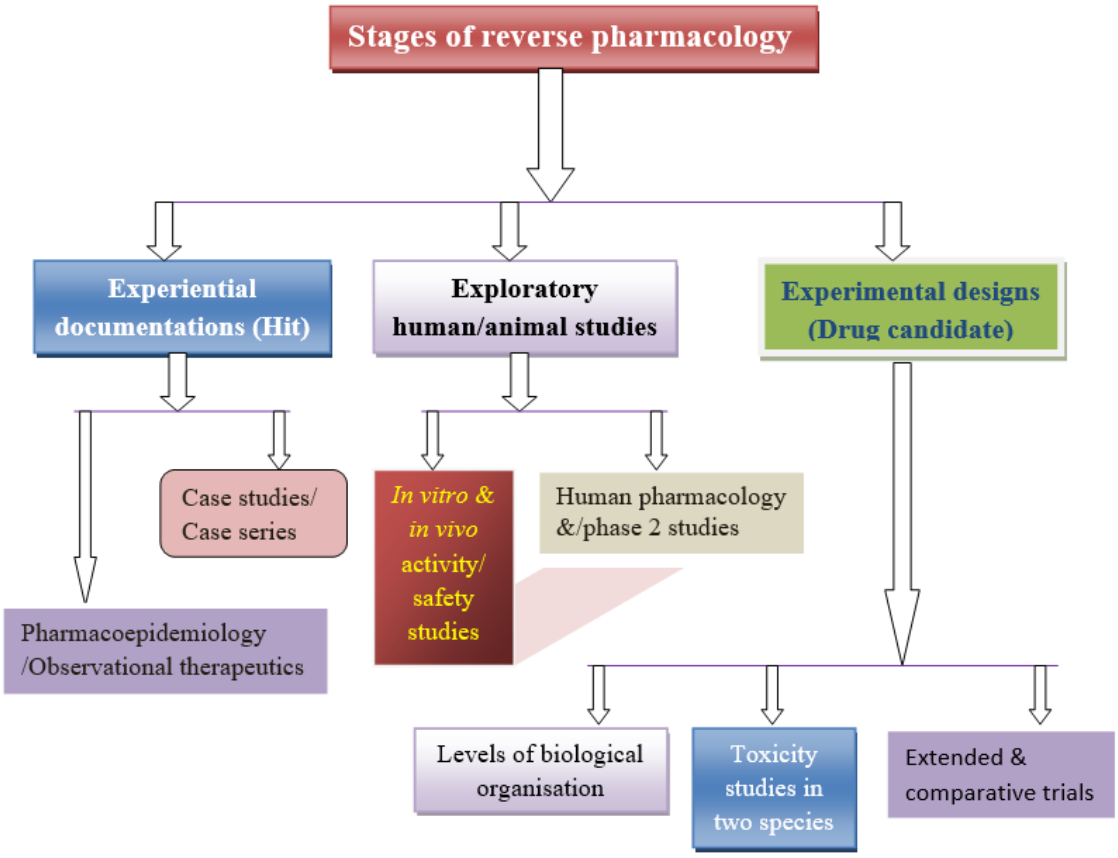

Figure 4: Stages of Reverse Pharmacology [28]

1.4. Novel Models In Reverse Pharmacology

Long pre-clinical and clinical trials must be conducted in a strictly linear manner as part of the conventional drug discovery and development process for NCE. The RP route, on the other hand, is a drug discovery and development circular model. It begins with the recorded human experience of TM. Next, a small sample of patients using a standardised formulation and objective end-points for safety and activity can start a dosage-searching study. In order to understand the product's drug-like activity and mechanism of action, concurrent in vitro and in vivo research might be initiated. It might even be necessary to develop entirely novel models that are comparable to the therapeutic outcomes seen at the bedside [30]. Using epistemology-sensitive research techniques is becoming more difficult as a result of this circumstance. The fundamentals of validity must be considered while using research and statistical techniques responsibly. The internal and external validity of evidence-based medicine (EBM) must be considered, with a focus on rational study designs and the capacity to generalise the results. In light of Ayurveda's theoretical underpinnings and historical practice, three forms of validity—consensual, congruent, and concurrent need to be taken into account. Congruent validity is the examination of the phenomenon at several levels of biological organisations; concurrent validity is the simultaneous evaluation of the biological plausibility of Ayurvedic descriptions and data from the biomedical sciences. Consensual validity is the agreement between practicing Vaidyas. The RP approach is developing to promote integrative management using drugs and non-drug measures by carefully balancing drug-targeted screening with personalised natural medicine [33,34].

1.5. Application of Reverse Pharmacology in Drug Discovery From Natural Products

1.5.1. Anti-Malarial Drugs

The best instance of the RP approach is presented by the discovery of artemisinin as a treatment for malaria, which is the outcome of a scientific study based on knowledge from TCM. Chinese TM has been treating malaria using Artemisia annua for many centuries. For the treatment of uncomplicated malaria, an RP approach is offered. It was employed in the preparation of Argemone mexicana, a TM used in Mali to treat malaria. In Mali, Argemone mexicana proved to be the most successful traditional remedy for treating uncomplicated falciparum malaria, with a decoction that was as clinically efficacious as artesunate. Selecting a treatment for development through a retrospective treatment-outcome study was the first step. A dosage-escalating clinical experiment that demonstrated a dose-response phenomenon and assisted in determining the safest and most effective dose was the second step. A randomised controlled study was the third step,

in which the phytomedicine was compared to the conventional first-line treatment. Finding active molecules that can be used as markers for standardisation and quality control was the last stage. This RP example demonstrates that standardised phytomedicine can be produced more quickly and affordably than conventional medicines. The effectiveness of both approaches in terms of public health and their complementarity should be carefully evaluated, even though they are not entirely comparable [28,35].

1.5.2. Anti-Parkinson's Drugs

Parkinson's disease was initially treated using Mucuna pruriens seeds by Ayurvedic doctors in ancient India. When compared to synthetic L-dopa, the dosage utilised by ayurvedic doctors is far lower. These findings prompted researchers to carry out additional research and resulted in a partnership between academic institutions and Mumbai-based Zandu Pharmaceuticals. This group studied mucuna in order to discover a natural Parkinson's disease medicine. Clinical study new drug applications (NDAs) have been approved by the US Food and Drug Administration (USFDA). The Indian FDA has finally approved Zandopa. This natural product is standardised, safe, and affordably priced, making it an excellent substitute for synthetic L-dopa formulations [28].

1.5.3. Anti-Hypertensive Drugs

Reserpine, the anti-hypertensive alkaloid from Rauwolfia serpentine, is the best example of bio-prospecting using traditional knowledge. It was made possible by research done in close collaboration with Ayurvedic specialists by CIBA Pharmaceuticals in India [28].

1.5.4. Other Drugs

Guggulipid is a cholesterol-lowering medicine that was developed based on the principles of Ayurveda from Commiphora mukul. A memory booster produced via the RP approach from Bacopa monnieri. Lupin Laboratories in India attempted to develop an oral herbal formulation based on a single plant using the RP technique as part of the NMITLI project. A single plant's herbal beneficiated extract, called Desoris, is a promising treatment candidate since it efficiently modifies cellular activity, improving psoriatic lesions through a unique mode of action [4]. The Tanga AIDS Working Group (TAWG) in Tanzania has creatively applied traditional knowledge to lessen HIV/AIDS-related suffering. With the help of plants recommended by local healers, the group has successfully treated over 4,000 AIDS patients. The US National Institutes of Health and the Tanga AIDS Working Group have partnered to work on the scientific confirmation of the effectiveness of these herbal remedies, thanks to a cooperation that was started by the Global Research Alliance and the World Bank to expedite this process [31]. The pharmacological mechanism and material foundation of Resina draconis were studied in China using the RP approach. The synergistic action of three components (cochinchinenin A, cochinchinenin B, and loureirin B) was shown to be the analgesic effect of Resina draconis, and this typical case verified the RP methodology [36].

1.5.5. Drugs With New Indications

Novel responses to bedside observations on existing medicines should also be considered. Research on novel indications for established drugs can greatly benefit from the application of the RP methodology. Tight regulations were put in place after the thalidomide-induced phocomelia tragedy shocked the entire globe. However, the identification of an optical isomer's safe application in multiple myeloma has been a significant advancement. The medical literature is aware of a number of these instances, including the use of vitamin K2-7 to treat muscle cramps, hydroxyurea to induce foetal haemoglobin, aspirin to suppress platelet aggregation, and botulinum toxin to treat spasmodic torticolis. Many more chances to expand the indications of well-known medicines may arise from the ongoing attention provided at the bedside. The stigma attached to the use of known drugs for unauthorised indications can be overcome quickly by the application of RP to the problem [1].

1.6. What is Network Pharmacology?

A recently developed discipline known as NP attempts to comprehend how drugs work and interact with many targets. It uses computer power to systematically list a drug molecule's chemical interactions within a living cell. NP emerged as a key instrument in deciphering the complex linkages that underlie the interactions between the botanical formula and the organism as a whole. By permitting an objective examination of possible target areas, it also seeks to identify novel therapeutic leads and targets as well as to repurpose already-existing pharmacological molecules for various ailments. To choose the best targets and novel therapeutic molecule scaffolds, these efforts do, however, need some direction. Traditional wisdom can be quite helpful in the formulation, discovery, and repurposing of already-approved medicines. The next generation of promiscuous medicines could be intelligently designed by merging breakthroughs in systems biology and NP. NP analysis attempts to enhance the safety and effectiveness of currently available drugs in addition to providing new therapeutic options [36]. Through the integration of computational, experimental, and clinical research, NP connects scientists to contemporary science and technology while also enhancing understanding of the characteristics of traditional medications. NP is the result of several groundbreaking works.

1.7. Network Pharmacology Concepts and Significance

Network-based approaches are being used in research methods to develop novel medicines. Certain herbal products used in traditional therapy have shown promising results when these techniques are applied. NP is a contemporary technique for identifying the active ingredients and potential molecular targets in a range of herbal preparations or common plants [37]. As the information age progresses, different network technologies are being developed on an ongoing basis. NP, which combines pharmacology with information networks, is becoming more and more popular. It is based on system biology, bioinformatics, and high-throughput histology. The approach integrates network biology and polypharmacology. We can quickly find drug and illness

targets from a vast quantity of data using NP, and we are able to understand the interactions and pathways that exist between them. It's a useful approach. These days, the range of applications for NP is growing and includes investigating the basic pharmacological effects of medicines on illnesses and their mechanisms, analysing TCM theory, and exploring TCM application [38]. As a result of substantial developments in chemical biology, systems biology, and network biology, NP is swiftly emerging as a cutting-edge topic for modern drug discovery and development research. Because it combines computational and experimental techniques with reductionist and systems approaches, NP has the potential to be the next generation of drug discovery approaches. A new field of pharmacological study has emerged as a result of NP. Based, among other things, on theories of multi-directional pharmacology and systems biology, NP can construct complex network models to study the biological or pharmacological properties of a target and explore its physiological or pharmacological mechanism through the use of high-throughput data analysis, virtual computing technology, and network public databases. NP is a useful bioinformatics approach for identifying all potential bioactive component targets, functions, and mechanisms in order to cure disease [39].

Common tools of NP include databases like DrugBank, STITCH, and TCM chemical information databases containing data on drug molecules, databases related to active ingredients such as PubChem and ChEMBL, target databases (KEGG, HIT, TTD, and PDTD), gene-related databases like OMIM, BioCarta, GeneCards, PharmGBK, and SwissTargetPrediction, protein-related databases like HPRD, BioGRID, InterPro, UniProtKB, PDB, and DIP, pathway analysis databases such as NetPath, Reactome, SignaLink, and MetaCoreTM, databases related to chemical structures such as CB, ChemSpider, LookChem, and MMsINC, and biomolecular interaction databases like HPRD, BIND, DIP, HAPPI, MINT, CPDB, STRING, and PDZBase. They can all be used to find the necessary information. Besides these databases, appropriate tools are needed, such as Cytoscape, Pajek, VisANT, GUESS, WIDAS, PATIKA, PATIKAweb, Ucinet, NetworkX, NetMiner, and CADLIVE. At present, in the field of TCM research, Cytoscape, GUESS, Pajek, and VisANT are the most widely used network analysis software [5,38,40-43].

The control of the signalling pathway with numerous channels, improvement in therapeutic efficacy, decrease in side effects, improvement in clinical trial success rate, and reduction in drug discovery expenses are some of the benefits of NP. Multiple genes and functional proteins interact in many complex illnesses [44]. The goal of NP models is to provide answers to problems like how and where a particular target in the illness network suppresses or activates disease phenotypes. By addressing the illness network at the system level through synergistic and deadly interactions, this ideally results in therapies that are less susceptible to drug resistance and have fewer adverse effects. Therefore, the regulated pathology network should be investigated by drug discovery methodologies in order to lower the disease networks' generally high attrition rates. Numerous studies have documented the intriguing biological insights gleaned from these networks, and a meta-analysis based on illness gene networks identified over 40% of the drug-activated targets linked to multiple disorders. Consequently, NP can help reduce the high failure rates in discovery efforts by aiding in the systematic characterisation of pharmacological targets [45,46].

1.8. Scope and Objectives of Network Pharmacology

Due to advancements in systems biology, NP has led to a significant transformation from "one-target, one-drug" approaches to "target-network, multi-component therapeutics." The scope of NP research includes, but is not limited to, the following areas [47-50]:

(1) Emphasizing diverse computational techniques, such as network-based and machine learning methods, and the various sources proposed and utilized throughout the research process. Numerous computational bioinformatics methods have been developed and applied across different aspects of NP.

(2) NP incorporates all the mentioned criteria to enhance the effectiveness and safety of proposed drugs and their powerful combinations.

(3) Network analysis is used to map protein-protein interactions and synergistic effects.

(4) NP studies involve computational and statistical methods for biological network analysis, with a focus on chemotherapeutic agents and TCM.

(5) Bioinformatics is being leveraged in NP to advance drug discovery by deepening our understanding of pharmacological effects.

(6) The objective is particularly significant as access to more advanced omics platforms will shape future research phases, shifting the focus from data collection to integrative data analysis through a network perspective.

1.9. Network pharmacology and Traditional Chinese Medicine

There is a strong correlation between the history and progress of NP and TCM research. NP is a distinct medical system that was developed based on traditional experience and differs significantly from the "drug-target-disease" theoretical framework of contemporary medicine. Combination therapy can be studied naturally because TCM experience places a strong emphasis on the concepts of "diagnosis and treatment" and "holistic view" in the treatment of diseases. In particular, Chinese herbs are typically used in compounded preparations to develop TCM formulas that adhere to the combined therapy principles of "Jun Chen Zuo Shi" and "the seven methods in prescription compatibility". TCM has increasingly been more globalised in recent years, and the modernisation of TCM has emerged as the primary concern of TCM research, which requires scientific validity. Nevertheless, it is challenging to understand the molecular mechanisms of TCM components due to the complexity of TCM compounds and their intricate interactions with biological systems. In order to standardise TCM, it is imperative that the molecular mechanism, bioactive markers, and toxic mechanism of the treatment be

identified. Although NP is a systematic biological tool for the research of multi-target and multi-pathway pharmacological effects, TCM treatment is a multi-compound, multi-targeted, and integrative paradigm. As a result, NP has gained popularity as a method for examining TCM's therapeutic mechanisms, and it has proven to be effective in determining the science behind TCM. Some strong initial progress has been made using the NP approach in examining the fundamental properties of TCM and identifying its numerous effects with multiple targets, multiple pathways, and multiple components (Table 2). Together, NP and TCM share a fundamental concept and support one another [27].

S. No.	Herb/Formulation	Disease treated
1	Phyllanthus emblica	SARS-CoV-2
2	Bioflavonoids	COVID-19
3	Saikosaponin compounds	COVID-19
4	The Genus Terminalia (Combretaceae)	Anti-cancer, anti-HIV-1, anti-fungal, anti-bacterial, anti-malarial, anti-oxidant, diarrhoea, and analgesic bioactivities *in vitro* or *in vivo*
5	Piper longum	Neurological disorders
6	Triphala	Gynecological cancers

Table 2: Some Examples of the Herbs in Network Pharmacology [39].

1.10. Network Pharmacology Research Techniques

The advancement of various research technologies, such as computer technology, omics technology, high-throughput technology, network analysis technology, network visualisation technology, molecular interaction technology, etc., has been closely linked to the development of NP in recent years. The use of these technologies helps to increase the prediction models' accuracy in NP research. Additionally, NP-omics technologies, such as metabolomics, proteomics, and genomics, among others, can be more useful in observing how drugs function at different levels of regulation and control. Moreover, network visualisation technology, like guess, can enhance the construction of biological networks by reflecting the network's entire process and making it more visually appealing and intuitive. The complex connections and vast amount of information in the biological network are transformed into a more recognisable and intuitive visual network, which effectively reduces the difficulty of textual information and facilitates further research. Network analysis technology can also perform technical analysis on the established network, obtain useful information more effectively, and improve efficiency. Technologies that can directly depict the interaction between drug molecules and proteins include biofilm interference, plasma resonance, and nano-liquid chromatography-mass spectrometry. As long as the tissues and cells are intact, high-throughput technology can identify several detection signs at once and offers the benefits of visualisation along with strong real-time performance. The logical application of computer technology can assist in quickly reducing the vast libraries of compounds into a subset with limited resources and offer theoretical direction for future clinical and therapeutic use. Computational prediction serves as the link between experiment and theory. Through transcriptomic analysis of gene expression following Ma Xing Shi Gan Decoction (MXSG) administration in a rat model of LPS-induced pneumonia, Yang et al. combined computer science with NP and proposed that the thrombin signalling pathway and the Toll-like receptor (TLR) are essential pathways for MXSG-mediated anti-inflammatory effects. These NP research tools are still in their infancy and require more investigation and inquiry, despite their successes and significant convenience [51].

1.11. Research Approaches/Strategies of Network Pharmacology

The procedures of NP focus on the following steps:

- Mapping the disease phenotypic targets and the drug targets together in the biomolecular network. Establish a pragmatic network model and predict the drug target based on public databases or available data from earlier research.
- Establishing the mechanism of association between diseases and drugs. The mechanism of functional drugs should be explored for the network equilibrium principle.
- Analysing the network to dissect the mechanism between network targets and system regulation. Network targets are the key ideas of NP, which assumes that disease phenotypes and drugs act on the same network, the same pathway, or even the same target, thus affecting the balance of network targets and interfering with the phenotype at all levels [27].

NP-based approaches that consider the enormous complexity of various networks involved in various disease states are the driving force behind a new concept in drug discovery. Generally, NP-based approaches can be divided into two categories: static networks and dynamic networks.

1.11.1. Static Network

The static network is made up of two primary topological components: entities, or "nodes," and relationships, or "edges," as shown in Table 3, Figure 5. The network's vertices, or nodes, stand in for various objects, including small molecules, diseases, proteins, genes, and molecular pathways, as well as any other

entity that interacts with the system being modelled. The pairwise connections that represent drug-target interactions, target-disease interactions, protein-protein interactions, or transcriptional regulation are called edges. Directions, weights, and other attributes can be displayed in edges when the data is accessible; as a result, knowledge of the hierarchy of effects will develop [52].

Network Characteristics	Definition	Biological Entities and Functions
Node	Basic component interacting (pair-wise) with other node(s)	Small-molecular (metabolic network) Genes (genetic regulatory network) Proteins (protein-protein network)
Edge	A relationship between the nodes	Connection may be physical, regulatory, genetic interaction Metabolic network: enzyme-catalyzed reactions Genetic regulatory network: expression data
Degree	Number of links to other nodes	Associated with topological robustness of biological networks i.e., small degree nodes are more "disposable" than hubs
Betweenness	Number of shortest paths that pass through each node	Important for finding non-hub crucial nodes or classifying hubs according to their positions in the network
Closeness	Number of links to the center	Only applicable to connected networks
Eigenvector	Influence of a node in a network	Assigning relative scores to all nodes in the network

Table 3: Important Topological Characteristics in Static Network [52]

A network's topological characteristics are important for comprehending and gaining access to the scalability, resilience, and performance of its protocols and applications. The local characterisation of networks is aided by the application of numerous fundamental topological features known as "centrality," such as degree, betweenness, closeness, and eigenvector centralities (Table 3, Figure 5). One of the most widely used centrality measurements is degree, which is a fundamental variable that represents the topology of scale-free networks. The number of edges that link to a node is known as its degree. A molecule that interacts with numerous other molecules—referred to as "hubs"—would have a high node degree. Node degree in scale-free networks follows a power-law distribution and tells us how much access a given node has to other nodes. Another widely used centrality metric that characterises the influential level of communication between node pairs is Betweenness [53-55]. Stated differently, the definition of betweenness is the quantity of shortest paths that traverse each node. Finding non-hub essential nodes or categorising hubs based on where they are in the network are crucial tasks. The closeness centrality, which is the reciprocal of farness, can determine how long it takes for information to go to a certain node in a network by measuring the path's length. Due to the uncertainty surrounding the distance between unconnected nodes, this function can only be used in networks that are connected. The impact of a node in a network is measured by eigenvector centrality, which is not limited to the shortest paths. Based on the connections to high-scoring nodes that contribute more to the problematic node score than low-scoring nodes, it can be used to evaluate the relative scores of all network nodes. Finally, this centrality measure may shed more light on the network's topological characteristics, which may be helpful in explaining illness treatments and directing the development of new drugs in intricate static network research [52].

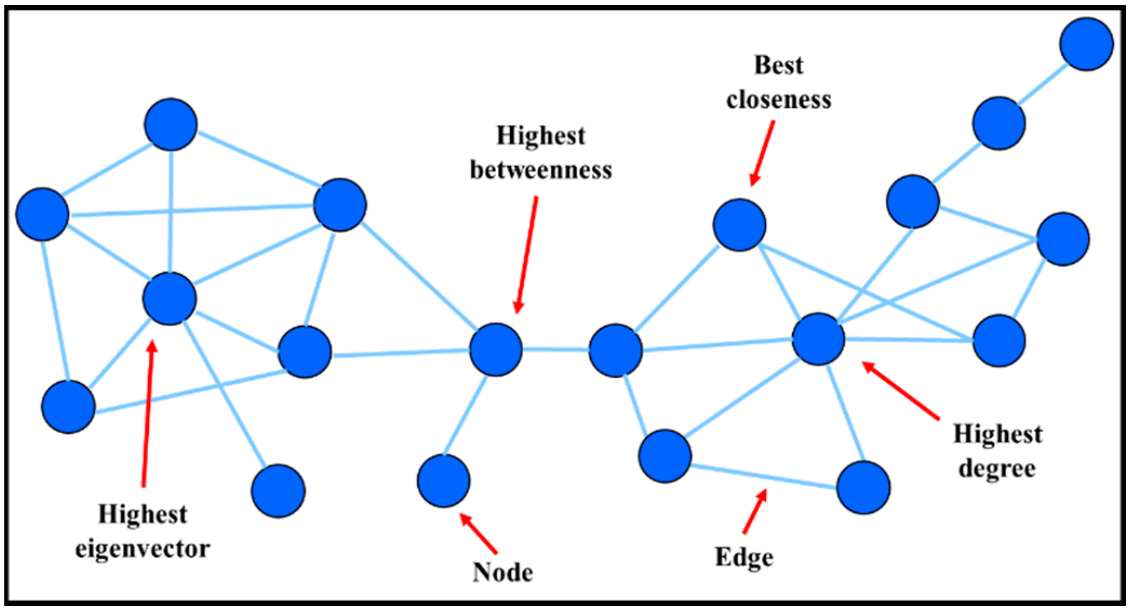

Figure 5: Topological Structure of Static Network [52]

1.11.2. Dynamic Network

Dynamic networks, in contrast to static ones, are those whose time-series structure is subject to alteration based on a variety of conditions. When compared to static networks, dynamic networks are more challenging since they require more data or data that is resolved spatially or temporally. Edge orientations, indications, conditionality, and several quantitative measurements that change dynamically are typically included in descriptions of dynamic networks. Dynamic network analysis and simulation offer a way to advance the discovery of new medicines by enabling us to better understand the dynamic behaviour of important players in space and time, as well as their function in human pathophysiology and the prediction of potential drug targets.

The modelling and simulation flowchart of a dynamic network is shown in Figure 6 [52].

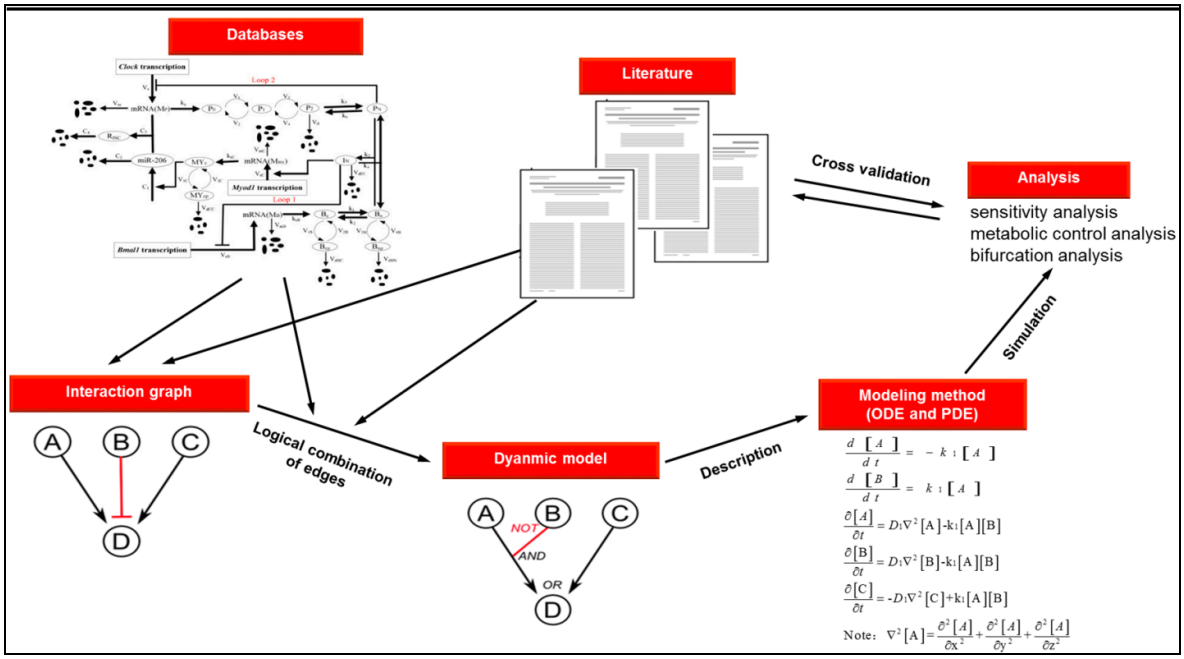

Figure 6: Modeling and Simulation Flow Chart of Dynamic Network [52]

Dynamic network modelling is commonly understood as a series of chemical reactions, the kinetics of which may be accounted for using partial differential equations (PDEs) and ordinary differential equations (ODEs) to do both stochastic and deterministic simulations. The most basic method for quantitatively simulating the dynamic reaction of each component in a network under various situations is to use a system of ODEs. This dynamic response can be determined analytically, either exactly or approximately. Deterministic ODE-modelled networks are easily able to explore both steady states and different dynamic behaviours with respect to the system's state. Even though compartmental ODE modelling is commonly used to simplify PDEs, precise spatial localisation might occasionally be crucial for cell signalling networks. In this instance, PDEs can be used to explain the diffusion and biochemical reactions of signalling markers in networks as reaction-diffusion equations in biochemical processes [56,57]. In these circumstances, a stochastic model based on PDEs that considers the intrinsic fluctuations in a dynamic network may result in qualitatively distinct behaviour that deviates greatly from the predictions of deterministic models. The reactant equations in the stochastic simulation were regarded as discrete entities, in contrast to the deterministic implementation. It is possible to explain stochastic fluctuations that could impact reaction dynamics. Because of the possible availability and repeatability of individual cell response variance, stochastic models are expected to be more widely accepted in light of the growing concern about the impact of signalling noise and the proliferation of single cell measurements [58].

1.12. Network Pharmacology Research Framework

Finding genes linked to compounds and diseases, building a network of protein–protein interactions (PPIs), and then assessing and visualising the network are the main goals of NP research. A straightforward place to start is by building molecular networks from massive databases. Next, important biological pathways are anticipated and important nodes are found using network analysis. Ultimately, further network validation is carried out to effectively verify the relationship between highly engaged constituents and their potential targets (Figure 7) [15].

1.12.1. Data Mining (Data Collection)

Finding the active ingredients in medicinal plants and disease-related targets is the initial step in NP research (Figure 7). Finding the active chemicals in a medicinal plant usually involves searching the literature, however many public databases offer an easy-to-use interface for this purpose. Following the collection of active compounds, publically accessible databases are consulted to obtain the canonical SMILES of those compounds. The detection of canonical SMILES may be accomplished with a few standalone and internet applications [59]. Gene prediction or from canonical SMILES is the focus of NP research after obtaining canonical SMILES. There are several easily navigable databases and tools available to help in the process. Another important thing to remember is that by applying a precise threshold to the probability of genes, we can obtain highly significant genes that are statistically significant. The first step in examining the molecular mechanisms of plants and herbs for treating a variety of diseases and disorders is the assessment of genes linked to disease [60]. The transcriptome-wide gene expression microarray profiles of isolated tissues in response to exposure to plant extracts, their combinations, or purified compounds have also been analysed by researchers, who no longer rely on data from the literature. This analysis is combined with novel pathway assessments conducted in silico to show the mechanisms of action and activated molecular networks behind estimated therapeutic effects in a variety of health conditions [15].

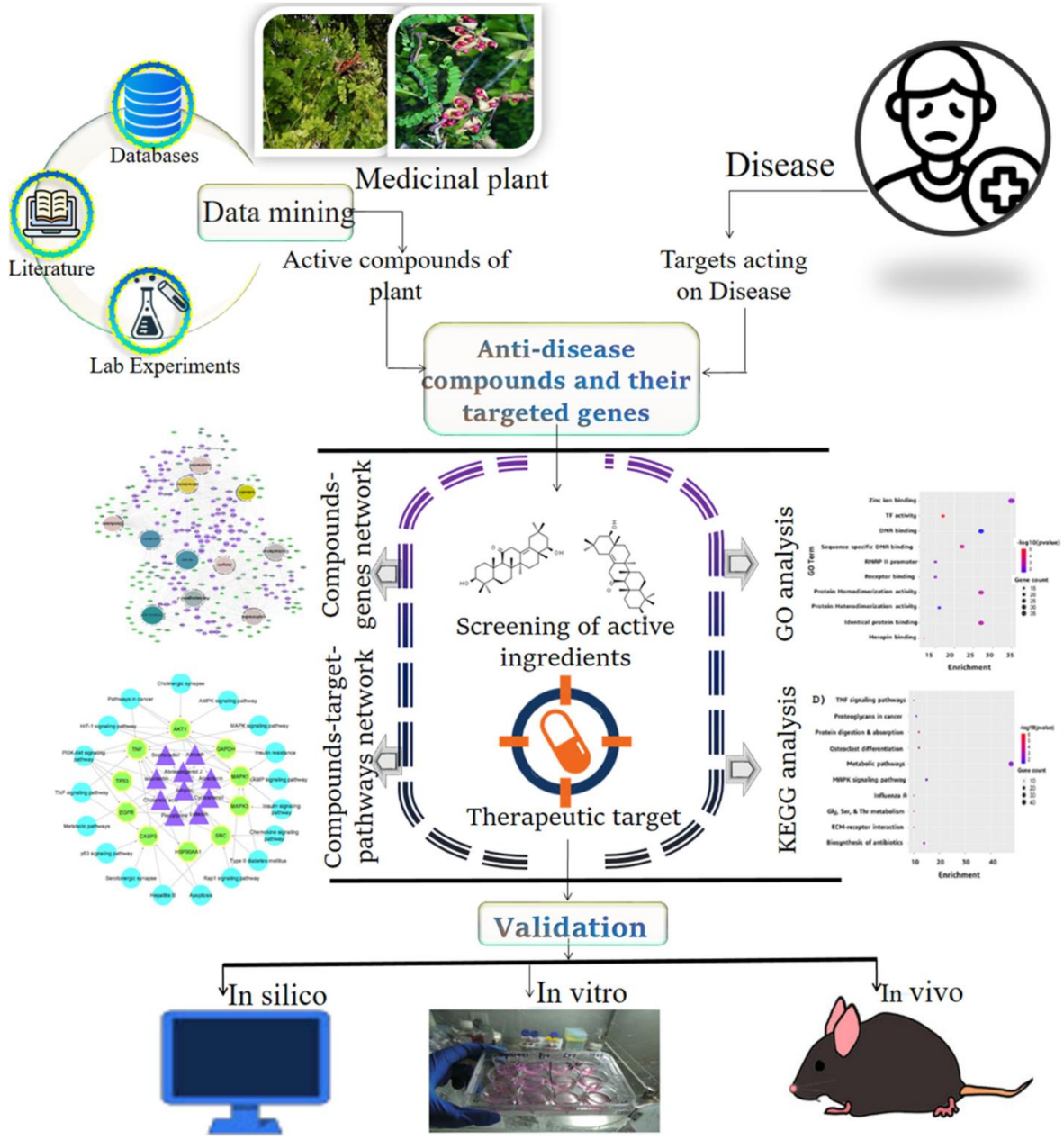

Figure 7: Graphical Synopsis Of Network Pharmacology Research For The Discovery Of Herbal Medicines Derived Targets, Effect Prediction, Mechanism Clarification, And New Drug Assistant Discovery Using Network Pharmacology Approach. It analyzes the information from public data, high-throughput experimental data, and herbal medicinal data and constructs network using technologies of network expansion, optimization, comparison, knockout, and addition. Finally, it carries out computational and experimental verifications [15].

1.12.2. Network Construction

A network is a diagram that shows how different entities, known as nodes, interact with one another. The nodes in pharmacological networks are disease, disease types, tissue, targets, bioactives, and pathways. The relationships between these nodes are represented by the edges—lines that connect them [61]. There are two opposing methods involved in building a network: a top-down method that begins with statistical analysis of the available data and a bottom-up method based on established biological knowledge (Figure 8). More specifically, there are multiple approaches to constructing and depicting a biological network. The de novo building of a network using direct experimental or computational interactions, such as chemical, gene, or protein screens, is arguably the most flexible and general method. An actual understanding of real biological events can be obtained using networks that include physiologically relevant nodes (genes, proteins, and metabolites), their connections (biochemical and regulatory), and modules (pathways and functional units) [62].

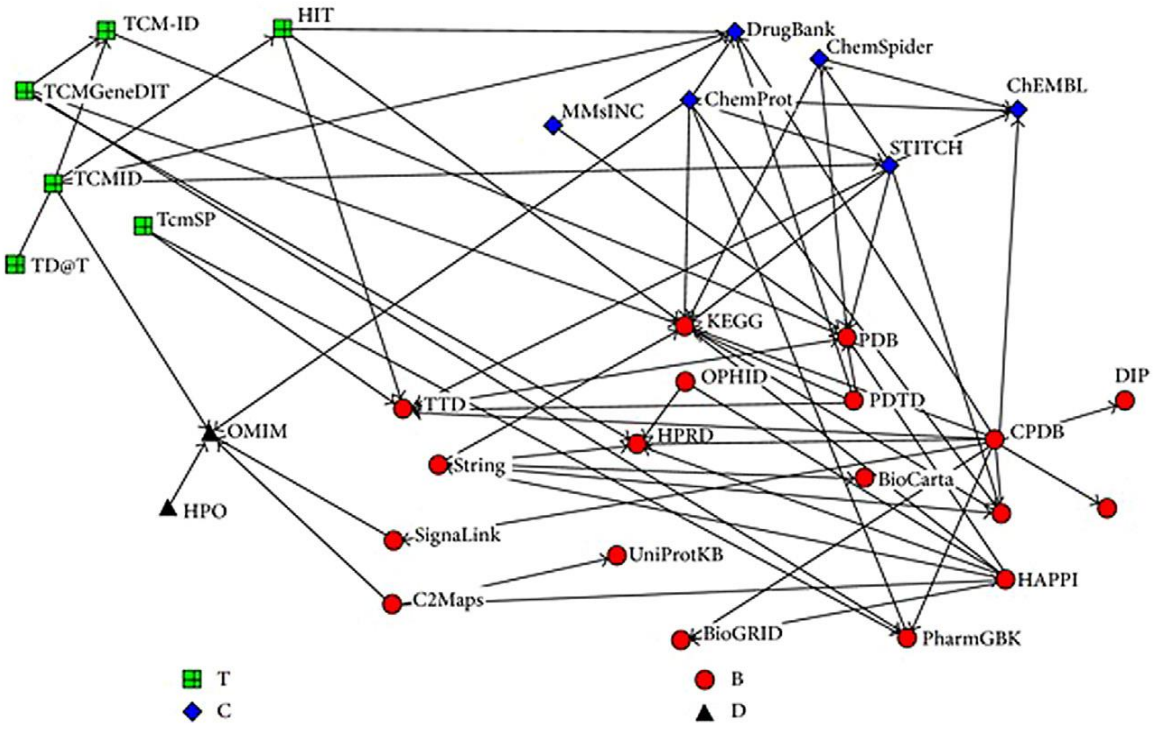

Figure 8: Database Relationship Network [37]

1.12.3. Network Visualisation

The process of obtaining interaction information from linked data and employing tools for analysis and visualisation is known as network visualisation. Networks can be classified into two main types based on the type of nodes they contain: single-element networks (Figure 9A) which contain nodes representing a single type of element (like PPI networks) and multi-element networks (Figure 9B) which contain nodes representing multiple types of elements (like drug-target-disease networks and drug-target-pathway networks). The variations in network properties affect not only the end result's visualisation but also the method of network analysis and the academic properties of the network's topology. The process of network visualisation involves two steps: (1) the creation of new network nodes, the linking between them, and the attributes assigned to them; (2) the network's description and the extraction of several tools to define the structural feature that clearly represents the network [27].

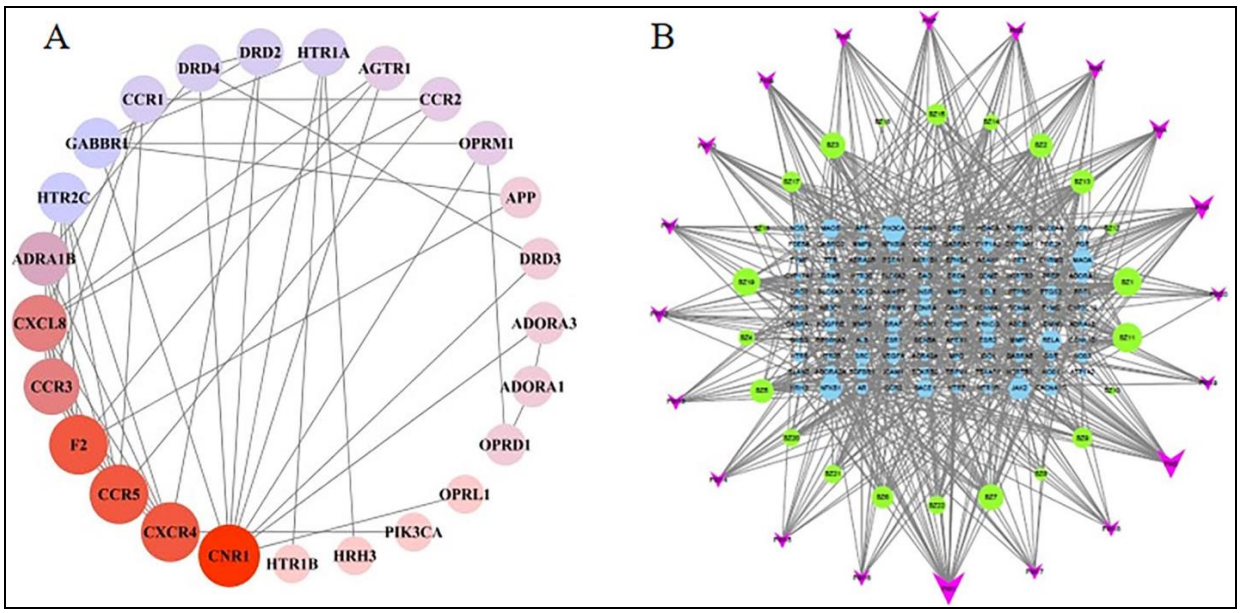

Figure 9: The Network of Different Categories.
A. Protein–Protein Interaction Networks Whose Nodes Are Protein.
B. Compound-Target-Signaling Pathway Networks Are Composed Of Compound Nodes, Protein Nodes, And Signaling Pathway Nodes (B) [27].

NP visualisation is frequently accomplished using specialised tools like Cytoscape. An effective tool for visualising molecular interaction networks and integrating them with any kind of attribute data is Cytoscape, an open-source software platform with a Java foundation. Apart from the fundamental functionalities for integrating, analysing, and visualising data, apps offer other features like molecular and network profiling analysis and connections to external databases. Many visualisation tools are available in addition to Cytoscape. Three main functional modules make up visual network pharmacology (VNP), which is specifically developed to visualise the intricate interactions between drugs, targets, and diseases. These modules are called drug-centric, target-centric, and disease-centric VNPs. This disease-target-drug database includes known relationships between targets, illnesses, and drugs approved by the US FDA. After conducting a search using terms related to diseases, targets, or drugs; chemical structures and substructures; or protein sequence similarities, users can get an interactive online network view of the records that they have found. The diseases, targets, or drugs are represented as nodes in the resulting network view, and the known connections between two of them are represented as edges [63].

The technique called Connectivity Map, or CMap, enables one to compare gene-expression profiles. The identification of the small molecule's mode of action may result from the similarities or variations between the transcriptional response profile of the small molecule and the hallmark transcriptional expression profile. The closeness of transcriptional responses between the response profile and drug response profiles in the CMap database is also compared. To have a better understanding of the mode of action, a network is built, and medicines that seem to be closest to the small molecule are chosen. Other programs that are useful for creating networks include Cell Illustrator, a Java-based program that specialises in biological processes and systems, and Gephi, an exploration platform for networks and complex systems [37]. When determining which diseases and medicines have overlapping targets, Venn diagrams are a better tool. The major goal of this step is to anticipate genes associated with diseases and then find the genes that are shared by chemicals and disorders. The initial benchmarks for additional screening are the common genes [15].

1.12.4. Network Analysis

Network analysis is used to comprehend how medicinal plants work to alleviate illness. PPIs' great adaptability, specificity, and versatility make them extremely important. Databases that offer functional connections between key targets are used to obtain the PPI network of important targets (common genes). The optimal degree of connection of the hub genes is predicted later using network analysis. Biological networks provide us with an abundance of data. The method for extracting vital information from networks is crucial [64]. By identifying targets, network analysis seeks to identify significant targets, active components, and the pathways that lead to them. Network structure analysis, network function analysis, and network analogy analysis are some of the approaches used in network analysis; network functional analysis is the most often used. It has been found that biological

networks possess a modular component, and numerous beneficial medicines function by modifying multiple proteins rather than just one. Through topological research, several sub-networks with distinct topologies and roles inside larger, more complex networks have been revealed. By examining their related pathways, KEGG pathway analysis and GO enrichment analysis offer unique and important target properties at a functional level [27,65].

1.12.5. Validation of Results

There are multiple techniques for validating the effectiveness of molecular targets that are expected. The most practical approaches are usually thought to be in vitro and in vivo; nevertheless, these procedures are expensive and time-consuming to get accurate findings. However, a number of in silico techniques have been developed with the advent of high-throughput technologies and the genomic era, offering a convenient platform for result validation. In general, there are virtual and experimental ways to verify the anticipated results [66]. Key targets and active compounds' docking sites are predicted using receptor-ligand molecular docking, which is based on NP. Consequently, mechanistic investigations on the synergistic effects of herbal remedies are substantially facilitated by NP and molecular docking, which effectively bridge the gap between western medicine and herbal medicine [67]. Microarray measurement of gene expression is another reliable method for validating the anticipated results. Thousands of genes are measured simultaneously by gene expression microarray analysis, which offers a comprehensive understanding of cell processes. These profiles can be used to show how cells react to a particular drug or to distinguish between cells that are actively dividing. One of the most effective and adaptable techniques for examining the expression patterns of a large number of genes in many tissues or within a single tissue under varied experimental conditions is high-density microarray technology. Large volumes of data are produced by the common use of microarray technology, which promotes the advancement of analytical techniques to predict the activity of target genes. Gene expression data are acquired from Gene Expression Omnibus (GEO) in order to analyse the varying gene expression levels of potential targets [68].

In several NP investigations, researchers use microarray analysis to confirm their predictions. Following a successful microarray study, real-time polymerase chain reaction (RT-PCR) is used to confirm the target genes that were found to be differentially expressed. These days, RT-PCR is a tried-and-true technique for identifying and measuring target genes in clinical diagnosis and therapy. A significant use of this technology as a research instrument is the quick and precise evaluation of alterations in gene expression brought on by disease, physiology, and development. Another indisputable and trustworthy method for confirming the target genes' expression levels is western blotting. Western blotting is a frequently used method by researchers to validate the outcomes obtained from the target-pathway interaction network [69]. Finally, a number of integrated techniques, including proteomics, transcriptomics, genomics, metabolomics, and high-throughput screening (HTS), can be used to quantify validated data. HTS is a practical strategy for the contemporary world. HTS is a scientific experimentation technique that is applicable to many different domains, including chemistry and system biology, and is very helpful in the drug development field. HTS technology has the ability to quickly find billions of data samples and forecast how chemicals or compounds will affect particular biochemical pathways. Additionally, this dual and innovative high-throughput technology makes it possible to collect network data from trials and experiments, which in turn validates the accuracy of the network model. For example, Fakhari and Dittmer created the PCR chip technology for gene expression analysis. The method was shown to be appropriate for high-throughput research. Verifying the molecular interaction between networks is another approach. When it comes to comprehending drug activity mechanisms and validating the drug network or anticipated model, this approach offers an innovative perspective. It mostly consists of biolayer interferometry (BLI) and surface plasmon resonance (SPR) technologies, which can help scientists, learn how drugs interact with biomolecules. BLI and SPR approaches employ high-throughput, high-precision, and real-time detection [15].

1.13. Implications of Network Pharmacology for Therapy

A recurring question about the optimal drug development approach is raised by the inability of independent candidate medicines to advance from pre-clinical to clinical trials. Due to patients' inadequate awareness and validation of these targets, even using medicines that target well-established targets linked to reliable biological networks presents challenges. Consequently, NP takes on greater significance and is receiving a great deal of attention in contemporary medicine research. For example, pleiotropically active compounds that target several proteins and biological processes in networks relevant to cancer may be beneficial. Worldwide, people use herbal medicine treatments to maintain their health. Because of their diversity in structure, bioactivity, and lack of toxicity, herbal medicines are recognised as a major source of innovative drugs that stimulate effective drug research and garner international attention [70]. The paradigm of "one disease, one medication, and one target" is being replaced with "one disease, one drug, and numerous targets". NP studies the conditions under which a single target can suppress disease characteristics like cancer growth, leading to the development of side-effect-free therapeutics. The benefits of NP have been enormous in the field of drug discovery, especially when it comes to repurposing already-approved drugs [71].

Network-based methods enable computational drug design, which helps forecast drug toxicity and helps the drug molecule find its target binding site. NP offers new avenues for drug research that could prove more fruitful than using unproven traditional therapy. People were curious about this idea because it raised the possibility of more targeted medications with fewer adverse effects on healthy physiological cells. NP may offer viable strategies for strict target selection and the development

of distinct, multi-target active agents to address those. In the PPI network, highly connected sections are preferred since these nodes are thought to be the main focus during the disease state. Therefore, by concentrating on these nodes, we might succeed in our goal. Drugs, on the other hand, cannot inhibit all targets in a regulatory network. Only around 15% of the nodes in a given network are druggable [72]. Various methods can be explored to develop effective phyto-therapies using network data. If the active components within herbs or herbal mixtures are identified, they can be utilized, drawing inspiration from their traditional use in herbal medicine. Herbal formulations resemble multi-drug-targeted therapies. By applying selective polypharmacological strategies, active compounds can be used to create treatments that target multiple specific sites. Proteins that are non-essential in normal cells may gain therapeutic importance when linked within a cancer network, where their combined inhibition or elimination could lead to more effective, possibly synergistic, destruction of tumor cells. This approach aligns well with human physiological processes, offering significant therapeutic potential. One possible solution is to employ polypharmacology to disrupt entire disease-causing networks using botanicals or complex herbal extracts that target multiple sites, rather than focusing on individual proteins [71,72].

1.14. Application of Network Pharmacology

Single-targeted drug discovery may or may not be a successful strategy, given the sharp decline in novel therapeutic alternatives despite research and development in the pharmaceutical sector. As opposed to traditional drug discovery methods, NP techniques address the possibility of drugs targeting many proteins or networks involved in a disease, making them very valuable in such cases. Predicted drug-target disease network models are also constructed with the use of high-throughput screening and bioinformatics. By comparing a drug's interaction with its corresponding target model, these approaches aid in the exploration of the fundamental processes of pharmacological actions on biological networks. Recent developments in network biology have significantly reinforced our understanding of multifaceted path interactions. As a result, NP is strengthening its position in the management of the most serious illnesses and ailments. NP determines which PPIs are connected to the clinical results of specific illnesses and conditions. These days, scientists are using computer technology and multi-omics methods to accurately capture the human unified metabolic response in order to investigate an ever-growing range of complex diseases. A few unique uses of NP in the biomedical sciences are covered here.

Mainly, NP has applications in TM, pharmacology, and drug research [15].

1.15. The Applications of NP in TM Include

- Scientific evidence for use of TMs.
- Understanding the rationale of traditional formulations.
- Understanding the mechanism of action of TMs.
- Network-based designing and prescribing of plant formulations.
- Analysis of multiple bioactives, studying synergistic action.
- Botanical biomarkers for quality control.

1.16. The Applications of NP in Pharmacology Include

- To develop new leads from natural products.
- Understanding the mechanism of action of drugs.
- Determining the possible side effects of drugs.
- Predicting new indications.
- Predicting possible drug and drug interactions.
- Drug repurposing.

1.17. The Applications of NP in Drug Research Include

- Identifying novel drug targets.
- Reduced cost and time through in silico evaluation.
- Understanding the signaling pathway of disease types.
- Therapeutics for multigene-dependent diseases.
- Discovery of disease-causing genes.
- Diagnostic biomarkers.
- Studying drug resistance or antibiotic resistance.

The application of NP in TCM includes elucidating the biological basis of diseases and syndromes, predicting the targets of TCM, screening bioactive substances, and deciphering mechanisms [27]. Deciphering mechanism of action of TCM using NP is depicted in Table 4 below. Moreover, application of NP in TCM is displayed in Table 5 below.

Disease	TCM	Methods	Effects and Mechanisms
Type 2 diabetes mellitus	Rhizoma Coptidis	NP, Molecular docking, Experimental validation	IL6, VEGFA, & TNF could stably bind with all active compounds of Rhizoma Coptidis & Rhizoma Coptidis could inhibit the expression of IL6 & TNF-α & enhance islet cell viability
Type 2 diabetes mellitus	Silkworm excrement	NP combined with experimental verification	AMPK/PI3K/Akt signaling was an important way for the anti-type 2 diabetic activity of silkworm excrement

Nephrotic syndrome	Qingrekasen granule	Integrated metabolomics, NP	Promoted autophagy & anti-apoptosis via the expression of AKT1, CASP3, BCL2L1 & mTOR, thereby protecting podocytes & maintaining renal tubular function
Diabetic nephropathy	Yishen capsules	NP	The active constituents of Yishen capsules modulated targets or signaling pathways in DN pathogenesis
Recurrent respiratory infections	Improved Panax ginseng C. A. Mey (Ginseng)-Schisandra	NP	Modified Ginseng-Schisandra Decoction was able to treat RRTI primarily through acting in the signal transduction of some key nodes of cancer pathway and TNF pathway
Hepatocellular carcinoma	Zuojin pill	NP	The compound-target network included 32 compounds and 86 targets, whereas the target-pathway network included 70 proteins and 75 pathways
Pyrotinib-induced diarrhea	Shenling Baizhu powder	Gut microbiota, Metabonomics, NP	The regulation of inflammatory bowel disease, IL-17 signaling pathway, pathogenic Escherichia coli infection and cAMP signaling pathway, were involved in the therapeutic effect of Shenling Baizhu powder against pyrotinib-induced diarrhea
Blood-heat and blood stasis syndrome	Moutan Cortex	Pharmacokinetics, NP, Molecular docking	F2, F10, F7, PLAU, MAPK14, MAPK10, AKT1, & NOS3 were screened as targets regulated by raw Moutan Cortex for the treatment of blood-heat and blood stasis syndrome
Alzheimer's disease	Chuanxiong Renshen decoction	NP, UPLC-Q–TOF–MS, Molecular docking	The downregulation of CASP3 and EGFR were involved in the therapeutic effect of Chuanxiong Renshen decoction against alzheimer's disease
Hepatic steatosis	Hawthorn or semen cassiae	NP	Hawthorn/semen cassiae treatment lowered expression of PPAR-γ and GRP78, thereby ameliorating ER stress and hepatic steatosis

Table 4: Deciphering Mechanism of Action of TCM Using Network Pharmacology [27]

TCM Herb/Formula	Diseases	Conclusion of the research
Astragaloside IV	Diabetic nephropathy	Transition properties, and ability to suppress the Wnt/-catenin signalling pathway
Bushen Zhuanggu formula	Breast Cancer	Pharmacokinetic analysis with network pharmacology to investigate the effect
Cynarin	Hyperlipidemia	Usage to treat hyperlipidemia in the future
Danhong injection with t-PA	Thrombolytic therapy	Improve BBB disruption, and minimise infarction, brain edoema, and haemorrhage following ischemic stroke
Erigeron breviscapus	Cerebrovascular disease	Activity against CBVDs by regulating various pathways and interacting with multiple targets
Fufang Danshen formula	Cardiovascular	Association of 9 metabolites alter 42 cardiovascular genes linked to 30 disorders
Ge-Gen-Qin-Lian Decoction	Type 2 Diabetes	4-Hydroxymephenytoin as a new antidiabetic component enhancing insulin secretion in RIN-5F cells and decreased insulin resistance in 3T3-L1 adipocytes
Hedyotis diffusa Willd	Colorectal Cancer	Aid CRC patients by altering cancer, infectious disease, endocrine, immunological, and neurological system pathways
Jian-Gan-Bao	Liver disease	Study of hepatoprotective properties
Kushen Injection	Lung Cancer	Validation and prediction of mechanism
Liu-wei-di-huang-wan	Diabetic ketoacidosis	Minimize the risk of diabetic ketoacidosis
Ma Huang Tang	Bronchial asthma	Reduce the pathological alterations of acute asthma like syndrome
Naoxintong	Cardiovascular & CBVDs	Benefits on angiogenesis and blood flow recovery
Panax notoginseng	Cardiovascular disease	Multidrug combination therapy for cardiovascular
disease is a possibility.		
Qingfei Paidu Decoction	COVID-19	Rreatment of COVID-19 in various stages
Realgar-Indigo naturalis	Promyelocytic leukemia	Examine the possible therapeutic impacts of formulae
Shen-qi-Yi-zhu Decoction	Antigastric, Cancer	Systematic technique for elucidating SQYZD's anti-GC mechanism
Taohong Siwu decoction	Osteoarthritis	Potential to prevent diseases like OA in a holistic and integrated way
Xuesaitong injection	Antimyocardial infarction	Identified 70 prospective XST targets
Yanghe Decoction	Breast cancer	Acts on HER2-positive breast cancer,
Zhi-zi-da-huang decoction	Alcoholic liver disease	Effective therapeutic method to treat alcoholic liver disease

Table 5: Network Pharmacology Applications in TCM [39]

1.18. Limitation and Solution

NP has proved to be beneficial in drug development, which aids in revitalizing herbal medicines. Albeit there are a few limitations of using NP for studying TM that would hopefully get resolved in the future. The major limitations and possible solutions are listed:

a. NP currently relies on various databases for literature and bioactive mining. Databases, though curated, may show discrepancies due to numerous sources of information, theoretical, and experimental data. Moreover, the botanicals that undergo certain preparatory procedures during the formulation of the medicine may have its constituents that have chemically changed due to the procedures; like boiling, acid/ alkali reactions, interactions between the bioactives, etc. A way to navigate around this problem is to make use of modern, high-throughput chemical identification techniques like ultra-performance liquid chromatography electrospray ionization tandem mass spectroscopy (UPLC-ESIMS/MS). This technique will help to identify the exact bioactives or the chemical constituents of the formulation, and will enrich the subsequent NP studies. This is because the bioactives form the foundation of any TM network.

b. Absorption, distribution, metabolism, excretion, and toxic effects (ADMET) parameters associated with the bioactives/formulation when they are administered in the form of the medicine need to be considered in order to extrapolate in silico and cheminformatics data to in vitro and in vivo models. In silico tools that offer the prediction of these parameters can be depended on for this. But TMs are generally accompanied by a vehicle for delivery of the medicine. These vehicles, normally various solvents—water, milk, lemon juice, butter, ghee (clarified butter), honey—that alter the solubility of the bioactives, play a role in regulating ADMET parameters. Experimental validation studies are required to evaluate this principle of TM.

c. Target identification usually relies on a single or a few databases due to the limited availability of databases with free access. This can occasionally give incomplete results. Also, there may be novel targets waiting to be discovered that could be a part of the mechanism of action of the bioactives. To deal with this discrepancy in the network, multiple databases should be considered for target identification. Integration of databases serving similar functions can also be a solution for this problem. In addition to this, experimental validation of the target molecules using protein-protein interaction studies or gene expression studies will provide concrete testimony to the network predictions.

d. A number of TMs act through multiple bioactives and targets. Synergy in botanical drugs helps to balance out the extreme pharmacological effects that individual bioactives may have. The interactions of bioactives with various target proteins, their absorption into the body after possible enzyme degradation, their transport, and finally their physiological effect are a crucial part of TM. However, in vitro assays or in silico tools are unable to give a clear idea as to the complete and exact interactions in a living organism. NP is only the cardinal step toward understanding the mechanism of bioactives/formulations. But this gives an overview of the action of TM which can be used to design in vivo experiments and clinical trials. This saves time and cost of research and inventions.

e. It is observed that formulations are working by simultaneous modulation of multiple targets. This modulation includes activation of some targets and inhibition of other. In order to understand this complex synergistic activity of formulation, investigative studies regarding the interactions of ligands with targets are to be carried out. This can be achieved by implementing high-throughput omics studies based on the network data [15,36].

2. Conclusions

The high failure rates of therapeutic candidates at various stages of development raise concerns about the current drug discovery paradigm. Ayurvedic medicine offers a wealth of traditional knowledge that could be explored to develop new drugs. To investigate this experiential data further, relevant in vitro and in vivo studies can be conducted. The transdisciplinary approach known as RP shows great promise for creating innovative natural medicines and their derivatives, with encouraging progress already made. For RP to succeed, it requires regulatory support and acceptance. There is a need to reconsider novel human biodynamic effects and explore their therapeutic potential. Asia and Africa could contribute to enhancing integrated health care through RP. Techniques for drug development based on TM and natural products are becoming increasingly popular. Many pharmaceutical companies are now focusing their research and development efforts on producing botanical medicines by leveraging insights from traditional herbal therapies. Herbs provide significant chemical diversity and can help identify new therapeutic targets. The experimental and knowledge base of traditional herbal medicine offers novel, practical ways to address the challenges of toxicity, time, and cost, which are major obstacles in conventional drug development. TM-inspired RP is a practical and effective approach to discovering new treatment candidates. Advances in network biology, polypharmacology, and systems pharmacology have significantly progressed NP, highlighting future technological possibilities. This approach is increasingly applied in research on natural products like plants, herbs, and Ayurveda, which have been used for centuries without fully understanding their statistically significant effects. Instead of the current single-targeted approach, NP offers a solid scientific foundation for future research on multi-targeted outcomes. Recent studies aim to utilize large data sources, network construction, analysis, and visualization to drive successful development and ongoing advancements in drug discovery.

Future Perspectives

Treating a patient with a single medication may not be the most effective approach due to the unique combination of traits each person has, influenced by genetic diversity. A more prudent strategy might involve using genome-wide functional screening to target diseases. Integrating Ayurveda with functional genomics within a systems biology framework could clarify the pathways of key and active components. Pharmacogenomics is playing a significant role in drug discovery, and it is advisable to genotype drugs metabolized by enzymes with genes that contain inactivating polymorphisms. Research has found correlations between the three basic Prakriti types (the traditional Ayurvedic method of categorizing individuals) and both phenotype and genotype. This discovery opens up an exciting new area of research, leading to the development of personalized treatment approaches.

In the current global economic climate, combining innovative methodologies with traditional knowledge remains crucial for advancing research and injecting new energy into the field. Despite the great potential and promise, there are currently only a few notable success stories. It is widely understood that this research, although seemingly outdated, can only be fully leveraged by collaborating with the most skilled academic and industrial partners across public and private sectors. This somewhat reductionist approach focuses on isolating a specific molecular target, typically an enzyme, and searching for a small molecule to influence the target's known or predicted function. Consequently, RP offers a promising platform for innovative drug development. There are numerous opportunities to utilize conventional wisdom

through network pharmacological analysis to address the challenges currently facing the drug discovery industry. NP can significantly contribute to developing new drugs, repurposing existing ones, and creating rational formulations. Various experiments have investigated different combinations of bioactive targets, and NP synthesizes this data based on the bioactive components of traditional formulations, providing insights into their mechanisms of action. This approach infers the molecular mechanisms of these formulations by using modern integrated technologies in a reverse-engineering manner. Although the current network analysis relies on existing literature and research, and results are inconclusive due to ongoing studies and the continuous generation of new information, this method remains valuable. It helps uncover the deeper significance of our long-standing traditional knowledge. NP facilitates a logical analysis of this knowledge, which can be used to both understand the data and develop innovative solutions to pressing pharmaceutical challenges.

Future advancements in NP are essential to ensure its continued growth and effective use. This includes enhancing reliability, expanding and refining various databases, and integrating NP with other strategies and processes. Additionally, establishing comprehensive guidelines for NP analysis methods will help make the results more convincing and representative. To broaden the application of network analysis, new methodologies need to be developed. Quantification will also play a crucial role in verifying whether the active ingredients achieve the necessary pharmacodynamic concentrations. To validate NP findings, it is important to integrate them with research in pharmacology, pharmacokinetics, toxicology, and pharmacodynamics. When approached holistically, NP provides a promising opportunity to accurately address both the symptoms and the root causes of diseases.

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