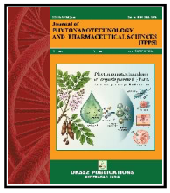
Journal of Phytonanotechnology and Pharmaceutical Sciences 6(3): 84-92, 2026

Online ISSN:2583-0376

http://jpps.ukaazpublications.com

DOI: http://dx.doi.org/10.54085/jpps.2026.6.3.9

Journal of Phytonanotechnology and Pharmaceutical Sciences



Review Article : Open Access

# Network pharmacology in modern drug discovery: Principles, computational approaches, network ethnopharmacology and experimental validation

**Fatima Umaira Saeed**◆

*Department of Pharmacology, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Hyderabad-500027, Telangana, India*

**Article Info**

**Article history**

Received 17 July 2026

Revised 29 August 2026

Accepted 30 August 2026

Published Online 30 September 2026

**Keywords**

Network pharmacology

Natural products

Drug discovery

Molecular docking

**Abstract**

Network pharmacology (NP) has emerged as a systems-level approach to drug discovery that addresses the limitations of the conventional single-target paradigm by investigating the complex interactions among compounds, targets, genes, proteins, pathways, and diseases. The development of network biology and the recognition of polypharmacology have provided the conceptual foundation for NP, particularly for understanding the multitarget actions of natural products and traditional medicines. Network ethnopharmacology (NEP) further extends this approach by integrating ethnomedical knowledge with computational and systems biology methods to investigate the molecular basis of traditional medicinal practices. This review describes the evolution of drug discovery from traditional medicine and target-based approaches to network-based strategies and discusses the fundamental principles underlying NP and NEP. The major stages of a typical NP workflow, including identification of bioactive compounds, database-based compound profiling, ADME and drug-likeness assessment, target prediction, disease-associated gene mapping, intersection analysis, network construction, topological analysis, pathway enrichment, and experimental validation, are discussed. The integration of molecular docking, pharmacokinetic analysis, artificial intelligence, multiomics approaches, and emerging structural prediction methods such as AlphaFold3 is also highlighted. In addition, the review discusses the application of NP in drug repurposing and the development of multitarget therapeutic strategies. Despite its potential, NP is constrained by limitations related to database completeness, methodological standardization, computational false positives, the complexity of traditional medicines, and limited translation of computational findings into clinical evidence. Future progress will depend on improved data quality, standardized workflows, integration of AI and multiomics data, and stronger experimental and clinical validation. Overall, NP and NEP provide complementary frameworks for connecting traditional medicinal knowledge with modern computational and experimental drug discovery.

## 1. Introduction

Drug discovery, which refers to the process of identifying new candidate medicines, initially relied largely on the traditional use of natural substances obtained from plants, animals, and naturally occurring minerals (Burger, 1964). Early practitioners depended on the Materia Medica developed and preserved by medicine men and priests based on their observations and therapeutic experiences. With the development of classical pharmacology, the approach gradually became more systematic, with potential therapeutic compounds being evaluated in intact cells and whole organisms to determine their biological effects. A major transformation in drug discovery occurred with the development of human genome sequencing, which contributed to the emergence of target-based drug discovery, also known as reverse pharmacology. This approach is based on the concept that modifying the activity of a specific protein can produce a desired therapeutic effect. Such proteins, with which drugs interact or to which they bind, are commonly referred to as drug targets. In this reductionist approach, compounds from chemical libraries are screened to determine their ability to influence the known or predicted function of a particular target (Hacker *et al*., 2009). Once a promising compound is identified for a specific target, its chemical structure can be further optimized at the molecular level to improve its interaction with the target and enhance its therapeutic properties. Consequently, drug discovery for several decades largely followed a "one drug-one target-one therapeutic effect" concept, focusing on the interaction between a specific molecule and a defined biological target.

The information technology revolution in the late 20th century brought significant changes to the field of drug discovery as well (Clark and Pickett, 2000). During this period, advances in omics technologies provided new approaches for investigating different stages of drug research and development (Buriani *et al*., 2012). The increasing availability of computational resources further transformed the drug discovery process by enabling researchers to assess the drug-likeness of newly designed or identified compounds and to perform ligand-protein docking studies. These computational approaches help predict how strongly a small molecule may interact with the 3D structure of a particular protein. In addition, various in silico methods were developed to predict important pharmacological

**Corresponding author: Ms. Fatima Umaira Saeed**

*Assistant Professor, Department of Pharmacology, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Hyderabad-500027, Telangana, India*

**E-mail: umaira.rbvrrwcp@gmail.com**

**Tel.: +91-9392301704**

Copyright © 2026 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

and safety-related properties of drug candidates, including absorption, distribution, metabolism, excretion, and toxicity, collectively referred to as ADMET properties (Van de Waterbeemd and Gifford, 2003; Clark and Grootenhuis, 2002). Although, these technological developments improved the ability to identify highly specific drug candidates, they also encouraged a predominantly reductionist approach focused on finding highly selective "magic bullets," which differed considerably from the holistic principles of traditional medicine. The limitations of this highly specific, target-oriented approach have become increasingly evident, particularly in the management of complex and multifactorial diseases. A major concern is the occurrence of adverse effects, while another is the limited ability of single-target therapies to address diseases involving multiple interacting biological pathways. These limitations are closely associated with the linear nature of the conventional drug discovery strategy (Chandran *et al*., 2017). Natural products have historically played an important role in drug discovery and development because of their greater chemical diversity and, in many cases, favorable safety profiles compared with synthetic compound libraries (Zimmermann *et al*., 2007). At present, more than one hundred natural product-derived leads are estimated to be undergoing clinical development (Harvey, 2008). Bioactive compounds obtained from traditional medicinal sources can provide valuable lead molecules and structural scaffolds for rational drug design. Unlike conventional synthetic drugs that are often designed to act on a single specific target, natural products generally influence multiple biological targets and pathways. However, advances in drug discovery have largely focused on developing highly specific, single-target molecules that imitate the activity of natural bioactives, rather than investigating the mechanisms underlying their synergistic effects or developing approaches to preserve and utilize these interactions from natural sources (Patwardan *et al*., 2015). As it is increasingly recognized that many diseases arise from the dysfunction of multiple proteins and interconnected pathways, targeting a single molecule may not be sufficient for effective disease management. Addressing multiple targets involved in a disease-associated metabolic cascade may therefore provide a more holistic therapeutic strategy. This has encouraged a shift from the conventional single-target, single chemical entity approach toward a multi-target and synergistic formulation-based strategy (Patwardhan *et al*., 2015). Consequently, researchers have renewed their interest in natural sources, returning to an area that played an important role in the early development of pharmacology. This renewed focus highlights the need to rethink the drug discovery process by combining traditional medicinal knowledge with modern technological approaches (Patwardhan, 2014).

Network Pharmacology is a relatively new field that helps researchers understand how drugs interact with multiple targets in the body (Hopkins, 2007). It uses computational methods to study and organize the different molecular interactions of a drug within living cells. NP has become a useful approach for understanding the complex relationships between herbal formulations and their effects on the body as a whole (Zhang *et al*., 2013; Berger and Iyengar, 2009). It can also help identify new drug candidates and therapeutic targets, as well as find new uses for existing drugs by exploring a wide range of possible targets (Kibble *et al*., 2015). However, selecting the most suitable targets and useful drug scaffolds remains an important challenge. Traditional medicinal knowledge can provide valuable guidance in developing new formulations and finding new uses for existing drugs. By combining systems biology with NP, researchers are able to develop the next generation of drugs that act on multiple targets (Cho *et al*., 2012; Hopkins, 2008; Ellingson *et al*., 2014). Overall, NP can help identify new treatment possibilities while also improving the safety and effectiveness of existing medicines.

## 2. Evolution from network biology to network pharmacology

### 2.1 Network biology

The post-genomic era brought rapid advances in computational biology, allowing researchers to analyze and interpret the large amount of biological data generated from different sources. One of the major goals of this period was to identify the molecules present in living cells and understand how they interact with each other. Since cellular functions depend on complex interactions among numerous molecules, understanding these networks became essential for explaining normal biological processes as well as disease development. These developments led to the emergence of network biology, which provides a framework for studying biological systems as interconnected networks rather than as isolated components. This approach offered a new perspective for understanding the complexity of cellular functions and disease mechanisms (Barabási and Oltvai, 2004). During the early 21$^{st}$ century, computational approaches were increasingly developed to construct biological networks and investigate the relationships between disease-related genes and phenotypes. One such approach was literature mining and microarray analysis (LMMA), which combined information obtained from scientific literature with microarray data to construct and refine gene networks (Li *et al*., 2006; Huang and Li., 2010). Initially, a broad network could be generated from relationships reported in the literature and subsequently improved using experimental gene-expression data. This approach helped researchers organize large amounts of biological information, identify important gene interactions, and better understand complex physiological and pathological processes. It also provided useful information for designing further experiments and studying disease-specific biological networks. The increasing availability of biological data also influenced pharmacology. Researchers began to recognize that drug action could not always be explained by the traditional "lock-and-key" concept, in which one drug interacts with one specific target.

### 2.2 Polypharmacology

Large-scale analysis of drug-target relationships revealed that many drugs can interact with more than one target, a phenomenon known as polypharmacology (Paolini *et al*., 2006). In simple terms, just as one lock can be opened by different keys, a single drug molecule may interact with several targets, while one target may also interact with different drug molecules (Chandran *et al*., 2017). The integration of network biology with pharmacological databases further strengthened this concept. Data from resources such as DrugBank (Re and Valentini, 2013) and OMIM (Hamosh*et al.,* 2005) were used to investigate relationships between drugs, their molecular targets, and disease-associated genes. When drug-target networks were first constructed, researchers expected to observe mainly separate drug–target relationships based on the conventional one-drug-one-target-one-disease model. Instead, these studies revealed extensive connections between drugs and multiple targets, providing evidence for the widespread nature of polypharmacology (Yildirim *et al*., 2007). The identification of these complex drug-target relationships highlighted

the limitations of the traditional single-target approach. The biological functions of cells are controlled by interconnected proteins and pathways, and therefore, a simple one-protein-one-function model may not fully represent the complexity of biological systems. Based on the connection between network biology and polypharmacology, Hopkins then introduced the term "Network Pharmacology" (Hopkins, 2007; Hopkins, 2008). NP represents a shift from the traditional highly specific "magic bullet" approach toward a more comprehensive, multitarget strategy for drug discovery. This approach may be particularly useful for complex and multigenic diseases, where several biological pathways are involved. It may also support the development of personalized therapeutic strategies in which drug combinations or formulations can be tailored according to specific disease conditions. The integration of network biology and polypharmacology may also help address two major causes of failure during drug development: insufficient efficacy and toxicity. In addition, network-based approaches can expand the range of biological targets that may be considered for therapeutic intervention. Based on these possibilities, Hopkins proposed NP as a new paradigm for drug discovery (Chandran *et al*., 2017). It has led to several possible strategies for developing multitarget therapies. The first involves using a combination of different individual drugs. Although, this approach can target multiple pathways, issues such as patient compliance and potential drug-drug interactions may limit its application. The second strategy involves developing multicomponent formulations containing several active ingredients. However, such formulations may present challenges related to metabolism, bioavailability, pharmacokinetics, safety, and consistency. The third strategy is to develop a single compound capable of selectively interacting with multiple targets. This approach may simplify dosing and development because a single compound can be evaluated through a more straightforward regulatory pathway. Metformin provides an example of a drug initially developed for type 2 diabetes that has also been investigated for potential anticancer effects (Leung *et al*., 2013).

### 2.3 Emergence of network pharmacology

Following the development of the NP concept, several computational methods were introduced to explore complex relationships between genes, proteins, diseases, and drugs. One such approach was CIPHER (correlating protein Interaction network and PHEnotype network to pRedict disease genes), which uses information from human protein-protein interactions, disease phenotype similarities, and known gene-phenotype associations to predict and prioritize disease-related genes (Wu *et al*., 2008). CIPHER can help identify established disease genes as well as potential new susceptibility genes. Such network-based analysis also helps researchers understand relationships between similar diseases and their underlying molecular mechanisms. A later method, CIPHER-HIT, used a hitting-time-based approach to measure the closeness between nodes in heterogeneous biological networks (Yao *et al*., 2011). This method can be applied to phenotype-genotype networks to identify genes associated with particular disease phenotypes. Similarly, network-based gene clustering has been used to identify groups of responsive genes within condition-specific networks, providing insights into physiological and disease-related responses (Gu *et al*., 2010). NP has also been applied to the identification of miRNA-based biomarkers. In these studies, networks connecting miRNAs with their target genes are constructed and analyzed in relation to specific diseases. Combining network analysis with literature-based information can help identify potential miRNA biomarkers (Lu *et al*., 2011). Another application involves constructing drug-gene-disease networks, in which information from drug and disease databases is integrated with gene-related data. Analysis of these networks can reveal relationships between multiple genes, diseases, and shared drug targets, thereby providing insights into new drug-disease associations and their possible molecular mechanisms (Zhao and Li, 2012).With the development of network biology, natural products gained renewed importance in drug discovery because of their extensive chemical diversity and the ability of nature to produce structurally complex molecules through evolutionary processes (Wetzel *et al*., 2011). At the same time, researchers began to investigate the principles underlying traditional medicine using computational approaches. These developments helped organize the large amount of information available on medicinal plants, their constituents, therapeutic uses, and biological activities. One of the early efforts was the development of a comprehensive herbal medicine information system containing data on more than 200 anticancer herbal formulations, approximately 900 ingredients, and around 8500 small organic molecules obtained from herbal medicines (Fang *et al*., 2005). Such databases provided a systematic platform for studying herbal medicines and helped establish a connection between traditional medical knowledge and modern molecular biology.

## 3. Network ethnopharmacology

The development of several botanical and traditional medicine databases subsequently encouraged researchers to apply NP to herbal medicines and their formulations. This approach led to the concept of NEP, in which ethnomedical knowledge is integrated with network-based computational analysis to investigate the mechanisms of traditional medicines (Patwardhan and Chandran, 2015).

## 4. Integration of molecular docking and pharmacokinetic analysis

### 4.1 Molecular docking in NP and NEP

Molecular docking also became an important component of NP and NEP. It allows researchers to investigate the possible interactions between bioactive compounds and molecular targets. A large database containing approximately 197, 200 natural product structures was constructed and docked against 332 target proteins associated with FDA-approved drugs, demonstrating the considerable overlap between the chemical space of natural products and that of approved drugs. Molecular docking-based NEP can therefore be useful for exploring how multiple bioactive compounds may interact with different targets involved in disease networks (Gu *et al*., 2010).

### 4.2 Integration of NP with pharmacokinetics

The integration of NP with pharmacokinetic analysis has also been proposed to better understand the active components and material basis of traditional formulations (Pei *et al*., 2013). The combination of NP with advanced analytical techniques, such as UPLC-ESI-MS/MS, has further improved the characterization of bioactive constituents and their potential molecular mechanisms in botanical formulations (Xu *et al*., 2014). Target-based analysis has also suggested that some botanical formulations may influence a broader range of biological targets than conventional drugs, highlighting their

potential for multitarget therapeutic action (Zhang *et al.,* 2014). Overall, NEP provides a systematic approach for connecting traditional medicinal knowledge with modern computational and experimental techniques. By identifying bioactive compounds, predicting their molecular targets, evaluating synergistic interactions, and experimentally validating their effects, network-based approaches may support the development of multicomponent and multitarget therapeutics derived from traditional medicines. NP can also be used to systematically document and analyze the clinical prescriptions used by traditional medicine practitioners (Li *et al*., 2015). By connecting bioactive compounds with their molecular targets, associated diseases, and clinical symptoms, traditional medicine networks provide a useful framework for investigating the underlying principles and therapeutic mechanisms of traditional medicine (Luo *et al*., 2015).

## 5. Key principles governing NP

### 5.1 Network-based systems analysis

It uses concepts from graph theory, where genes, proteins, metabolites, and drugs are represented as nodes and their interactions as edges, while network topology helps identify important or highly connected components. The approach also recognizes that diseases can arise from disturbances in interconnected biological subnetworks, often referred to as disease modules, rather than from a single abnormal target. By studying these modules, researchers can explore how multiple pathways contribute to disease progression and how simultaneous targeting of different nodes or pathways may reduce biological adaptation and resistance. Furthermore, the integration of multi-omics data, including genomics, transcriptomics, proteomics, metabolomics, and epigenomics, provides a broader understanding of complex biological systems and helps move pharmacological research from a conventional descriptive approach toward a more systematic and predictive understanding of drug action and therapeutic outcomes. The essential databases and computational tools are listed together with their functions in Table 1 (Saha *et al*., 2026).

### 5.2 Computational and experimental validation of NP findings

The validation stage of NP helps bridge the gap between computational predictions and experimentally observed biological effects. It generally combines molecular docking, molecular dynamics simulations, and experimental assays to evaluate whether predicted compound-target interactions are biologically plausible. Molecular docking is used to predict the binding orientation and affinity of a compound toward its target protein, while molecular dynamics simulations can provide further information about the stability and behaviour of the resulting complex. Commonly used tools include AutoDock Vina for relatively rapid and high-throughput semi-flexible docking, Glide and GOLD are used for advanced docking and scoring, and Molecular Operating Environment (MOE) for molecular modelling and pharmacophore-based analysis (Noor *et al*., 2022). The predicted binding affinity is generally expressed as binding free energy (ΔG) in kcal/mol, where more negative values indicate stronger predicted binding. As a commonly used computational criterion, ΔG < -5 kcal/mol orΔG < -7 kcal/mol may be considered indicative of favourable or high predicted binding affinity, respectively. However, these values should be interpreted together with binding interactions, molecular dynamics results, and experimental validation rather than being considered independent evidence of biological activity (Yang *et al*., 2025).

**Table 1: Essential databases and computational tools in NP**

| Category | Databases/tools | Function in NP pipeline |
|---|---|---|
| Gene expression/transcriptomics | GEO, Expression atlas, GTEx | Provides gene-expression data to examine genes that are differentially expressed or associated with a disease or biological condition. |
| Disease module/network analysis | NetworkAnalyst, Cytoscape, R packages | Helps identify disease-associated modules, clusters, and relationships among genes, proteins, and pathways within complex biological networks. |
| Pathway and biological information | KEGG, Reactome, WikiPathways | Provides information on biological pathways and molecular processes, helping to understand how identified targets participate in disease-related mechanisms. |
| Chemical-target-disease network construction | Cytoscape | Integrates compound, target, gene, disease, and pathway information to construct a network showing the relationships among these components. |
| Survival/clinical validation | TCGA, cBioPortal, GEPIA2 | Allows potential targets to be examined using clinical and genomic datasets, particularly for assessing their association with disease characteristics and patient outcomes. |
| Molecular dynamics simulation | GROMACS, AMBER, NAMD | Can be used after molecular docking to investigate the stability and dynamic behaviour of compounds or target complexes over time. |
| ADMET/Drug-likeness prediction | SwissADME, pkCSM, ADMETlab | Evaluates drug-likeness, pharmacokinetic properties, and potential toxicity of candidate compounds during the early stages of computational drug evaluation. |

## 6. Methodological workflow of network pharmacology

The way drugs are discovered and developed has gradually changed from the traditional "one drug-one target" approach to a broader concept in which one drug or natural product may act on several targets and pathways at the same time. This change is particularly important for complex diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases, because these conditions usually involve several interconnected biological processes rather than a single molecular abnormality. Therefore, studying only one target may not provide a complete picture of how a disease develops or how a treatment produces its effects. NP addresses this complexity by combining systems biology, bioinformatics, and computational analysis to explore the relationships between compounds, genes, proteins, pathways, and diseases (Cui *et al*., 2025). It is especially useful for studying natural products and traditional medicines, where several constituents may work together through multiple molecular targets and pathways (Muthuramalingam *et al*., 2024). More recently, the incorporation of artificial intelligence (AI) and multiomics data has further expanded the scope of NP, allowing researchers to analyse large biological datasets and obtain a more comprehensive understanding of drug mechanisms (Noor *et al*., 2022).

### 6.1 Finding and selecting potential bioactive compounds

The first step in an NP study is to identify the chemical constituents present in the selected natural product and determine which of them may have potential biological activity. The identified compounds are then screened using pharmacokinetic and drug-likeness parameters to select the molecules that are most suitable for further investigation. This initial step is important because the accuracy of the compound information directly affects the quality of the target prediction, network construction, and subsequent biological interpretation (Saha *et al*., 2026).

### 6.2 Identifying the chemical constituents

The chemical constituents of a natural product can be identified either from previously published literature or through analytical techniques such as GC-MS and UPLC-MS/MS.

### 6.3 Collecting compound information from databases

Once the compounds have been identified, information about their chemical structures, biological activities, and reported targets can be obtained from publicly available databases. Commonly used resources include TCMSP, PubChem, and the encyclopedia of traditional chinese medicine (ETCM). Other databases, such as IMPPAT, and BATMAN-TCM, can provide additional information on medicinal plants and their associated phytochemicals and biological activities. These resources make it possible to bring together information from different sources and prepare an initial list of compounds for further computational analysis (Mereczet *al*., 2025).

### 6.4 Screening compounds using ADME and drug-likeness properties

After collecting the available compounds, the next step is to narrow down the list by examining their absorption, distribution, metabolism, and excretion (ADME) properties (Mereczet *al*., 2025). This screening helps identify compounds that are more likely to possess favourable pharmacokinetic characteristics and reduces the number of molecules that need to be considered in subsequent analyses. Among the parameters commonly considered in NP studies are oral bioavailability and drug-likeness (Muthuramalingam *et al*., 2024). Oral bioavailability represents the proportion of an orally administered compound that reaches the systemic circulation. In many conventional NP studies, compounds with an OB $\geq$ 30% are selected for further analysis, this cut-off may vary depending on the study and its objectives. Tools such as SwissADME can be used to estimate different pharmacokinetic and physicochemical properties of candidate molecules. Drug-likeness is used to determine whether the physicochemical characteristics of a compound are similar to those commonly observed in established drugs. It can be evaluated using resources such as Molsoft and DrugBank, with a DL $\geq$ 0.18 being a commonly reported criterion in NP studies (Noor *et al*., 2022).

### 6.5 Using AI for molecular design and optimization

AI-based approaches are now being incorporated into the early stages of drug discovery to improve the design and optimization of potential drug molecules. Generative platforms such as chemistry can use machine-learning approaches to modify existing structures or generate new molecular candidates with potentially improved pharmacological properties and reduced toxicity. Thus, AI allows researchers not only to screen compounds that already exist but also to explore how their structures can be modified to obtain potentially better candidates (Cui *et al*., 2025).

### 6.6 Predicting the molecular targets

After the promising compounds have been selected, researchers need to determine which proteins or other biological targets these compounds may interact with. This process is commonly referred to as target prediction or target fishing. It is particularly important for natural products because a single compound can interact with several proteins rather than acting through only one target. Identifying these possible interactions helps researchers understand the polypharmacological nature of natural products and provides the basis for constructing compound-target networks (Noor *et al*., 2022).

### 6.7 Computational approaches for target prediction

Different computational methods can be used to predict the potential targets of a compound. These methods are broadly divided into ligand-based and structure-based approaches. Ligand-based methods make predictions by comparing the chemical structure or molecular features of a compound with compounds whose targets are already known. The underlying idea is that compounds with similar structural characteristics may interact with similar biological targets. In contrast, structure-based approaches focus on the three-dimensional structure of proteins and examine whether a compound can fit into a potential binding site. Inverse docking is one such approach, in which a compound is evaluated against multiple protein targets to identify proteins that may have suitable binding sites. Using these complementary approaches can provide a broader list of potential targets for subsequent network construction, pathway analysis, and molecular validation (Saha *et al*., 2026).

### 6.8 Target development levels

The target development level system is useful for understanding how much is already known about a particular protein target and how far it has progressed toward therapeutic development. The Pharos database groups protein targets according to the available

biological and clinical evidence. Targets classified as Tclin are already targeted by approved drugs and therefore have established clinical relevance. Tchem includes proteins for which small-molecule compounds are known to act, no approved drug currently targets them. Tbio refers to proteins whose biological characteristics have been studied but for which no known small-molecule modulators are available. At the least-characterized end, Tdark represents proteins about which very little information is currently available. This classification can be particularly helpful in NP and drug repurposing studies because it provides an indication of how well a predicted target has been investigated and where further research may be needed (Zahoránszky *et al.,* 2022).

## 7. Role of AI and alphafold3 in target prediction

The use of AI has added a new dimension to target identification by helping researchers analyse biological information that may be difficult to interpret using conventional approaches. One important development is AlphaFold3, which can predict 3D molecular structures and provide useful structural information for studying interactions between proteins and other molecules. This can be particularly valuable when working with proteins that have limited experimental structural information. AI-based methods such as graph neural networks (GNNs) can also examine the complex relationships between compounds, targets, and diseases by analysing them as interconnected networks. Such approaches may help identify important targets and possible synergistic relationships among different compounds. In addition to target identification, AI can be used for molecular design and optimization, helping researchers modify or generate compounds with potentially improved activity and reduced undesirable properties (Noor *et al*., 2022).

### 7.1 Disease-associated gene mapping and target intersection

After identifying the potential targets of the selected compounds, the next step is to determine how these targets are related to the disease being investigated. This requires collecting disease-associated genes from suitable databases and comparing them with the predicted targets of the compounds. The genes or proteins that occur in both sets are referred to as overlapping or intersecting targets and may represent important molecular points through which the compounds could influence the disease process. These common targets can subsequently be analysed using protein-protein interaction networks, functional enrichment, pathway analysis, and molecular docking to obtain a better understanding of the possible mechanisms underlying the therapeutic effects of the selected compounds (Saha *et al*., 2026).

### 7.2 Intersection analysis and Venn diagram mapping

Once the potential drug targets and disease-associated genes have been identified, they are compared to find the common or overlapping targets. These overlapping targets are important because they may be involved in the disease process while also being influenced by the selected compounds. A Venn diagram is commonly used to visually identify these shared targets and provides a simple way of narrowing down the list for further analysis. In more advanced studies, this analysis can be combined with multiomics factor analysis and gene-expression or transcriptional data to examine patterns of gene co-expression and their possible relationship with disease-related outcomes (Noor *et al*., 2022).

## 8. Network construction and topological analysis

After identifying the common targets, information obtained from compound profiling, target prediction, and disease-gene mapping can be brought together to construct a biological network. Rather than looking at compounds and targets as separate lists, network construction shows how they are connected and provides a broader picture of possible drug-disease relationships. These networks can then be examined to identify important proteins, compounds, and interactions that may contribute to the overall pharmacological effect.

### 8.1 Construction of different types of biological networks

#### 8.1.1 Protein-protein interaction networks

PPI networks are used to show how proteins interact with or are functionally related to one another. Interaction information can be obtained from resources such as STRING, BioGRID, DIP, and MINT. Among these, STRING also provides confidence scores for predicted interactions based on evidence from experiments, databases, and published literature, which can help researchers assess the reliability of the identified relationships.

#### 8.1.2 Compound-target-disease networks

A C-T-D network connects the selected compounds with their potential molecular targets and the diseases or conditions associated with those targets. Such networks are particularly useful for natural products because several compounds may act on multiple targets, while individual targets may be involved in more than one biological process or disease. This helps illustrate the many-to-many nature of drug-target interactions.

#### 8.1.3 Host-pathogen networks

For infectious diseases, NP can also be used to examine interactions between host proteins and pathogen proteins. These networks may help identify host proteins that interact with several pathogen components and could therefore represent important points for further investigation (Li *et al*., 2023).

## 9. Topological analysis and identification of hub targets

Once a network has been constructed, topological analysis is used to determine which nodes have important positions within the network. These measures help researchers identify highly connected or strategically positioned proteins that may be relevant to the biological mechanism being studied.

a. **Degree:** Degree indicates the number of connections associated with a particular node. A node with a large number of connections is generally referred to as a hub, as it interacts with many other components of the network.

b. **Betweenness centrality:** Betweenness centrality reflects how frequently a particular node lies on the shortest paths connecting other nodes. A node with high BC may therefore act as an important link between different parts or modules of the network.

c. **Closeness centrality:** Closeness centrality reflects how close a node is to the other nodes in the network based on the shortest paths between them. Nodes with higher closeness can potentially reach or influence other parts of the network more efficiently (Noor *et al*., 2022).

## 10. Pathway enrichment and functional analysis

After the important targets have been identified, functional enrichment and pathway analysis are performed to understand their biological relevance. The selected genes or proteins are mapped to biological processes, molecular functions, and signalling or metabolic pathways using appropriate databases and computational tools. This step helps explain how the identified compounds and their targets may contribute to the observed therapeutic effect and provides a biological interpretation of the network findings (Saha *et al*., 2026).

## 11. Experimental and computational validation

The final stage of the NP workflow is focused on checking whether the predictions obtained through computational analysis are supported by experimental evidence. This step helps connect computational findings with actual biological activity and usually involves a combination of molecular modelling and laboratory-based validation. Using both approaches makes it possible to determine whether the predicted targets and mechanisms are biologically meaningful rather than relying only on computational predictions (Li *et al*., 2023).

### 11.1 *In vitro* and *in vivo* experimental validation

Computational predictions should ideally be followed by experimental validation to determine whether the predicted biological activity can be observed under laboratory conditions. *In vitro* studies use appropriate biological models or assays to examine the activity of the selected compounds under controlled experimental conditions and can provide an initial indication of their biological effects. Where appropriate, promising findings can subsequently be evaluated using in vivo models, which provide information about biological responses within a complete living system. Thus, experimental validation complements NP and computational modelling by providing evidence that supports, refines, or challenges the predicted compound-target relationships (Noor *et al*., 2022).

## 12. Challenges and limitations

Although, NP has provided new ways to study complex drug-target interactions, several limitations still affect its reliability and practical application.

a. **Database quality and data completeness:** The accuracy of an NP study depends greatly on the quality of the information available in public databases. However, many databases do not contain complete information on compound-target interactions, and some reported interactions have limited experimental support. In addition, databases may not be updated regularly, meaning that recently published findings may not always be included. These limitations can affect the reliability of target prediction and subsequent network analysis.

b. **Lack of standardization:** Many NP studies currently follow similar workflows, including compound screening, target prediction, network construction, and enrichment analysis. Although, this provides a general framework, differences in databases, screening criteria, analytical methods, and validation procedures can lead to different results between studies. Greater methodological standardization and clearly defined reporting guidelines are therefore needed to improve reproducibility and allow meaningful comparison between studies.

c. **Complexity of traditional medicines:** The complex composition of traditional medicines presents another challenge. A single herbal preparation may contain numerous compounds that act on several targets and pathways simultaneously. In addition, many computational prediction tools have been developed mainly for small molecules, making it difficult to analyse larger and more complex constituents such as polysaccharides and other macromolecules. This limitation is particularly important when attempting to explain the complete pharmacological activity of traditional medicinal preparations

d. **False positives in computational prediction:** Computational screening can identify a large number of compounds with apparently favourable pharmacokinetic or drug-like properties. However, these predictions do not always correspond to actual biological behaviour. For example, compounds predicted to have good oral bioavailability or drug-likeness may not necessarily be absorbed or detected in the circulation at biologically relevant concentrations. Experimental and, where appropriate, in vivo studies are therefore required to confirm important computational predictions

e. **Limited translation to clinical practice:** A major limitation of current NP research is that many studies remain at the computational or preliminary experimental stage. Although, these studies can generate useful hypotheses about possible mechanisms of action, relatively fewer findings progress to well-designed clinical studies. More high-quality clinical evidence, including appropriately designed randomized controlled trials, is required to establish therapeutic efficacy, safety, and long-term clinical relevance (Wang *et al*., 2022 and Saha *et al*., 2026).

## 13. Future directions

The future development of NP is likely to depend on the integration of computational methods with increasingly detailed biological and clinical information.

a. **Integration of artificial intelligence and machine learning:** The combination of AI, machine learning, and bioinformatics is expected to improve the analysis of large and complex biological networks. These approaches can assist in identifying important targets, predicting drug-target interactions, recognizing disease-associated patterns, and generating new hypotheses from large datasets.

b. **Contribution to P4 medicine and expansion of drug repurposing:** NP may also contribute to the development of P4 medicine, which encompasses predictive, preventive, personalized, and participatory approaches to healthcare. By integrating molecular, clinical, and patient-specific information, network-based approaches may support more individualized understanding of disease and treatment responses. NP also has considerable potential in drug repurposing, where existing drugs are investigated for therapeutic applications beyond their original indications. By connecting known drugs with disease-associated targets and pathways, computational approaches can help identify possible new uses and prioritize candidates for further experimental investigation (Vithalkar *et al*., 2025).

c. **Development of TCM-specific parameters:** For studies involving TCM, future databases could incorporate additional traditional medicine characteristics alongside molecular

information. Parameters such as "four qi and five flavors," meridian associations, and the "monarch, minister, assistant, and guide" principles could provide additional layers of information for analysing traditional formulations (Yang *et al*., 2025).

d. **Development of standardized validation approaches:** Future studies would benefit from standardized evaluation procedures that combine computational prediction, experimental validation, and clinical evidence. The adoption of appropriate methodological guidelines, such as the NP evaluation method guidance, could improve the reproducibility and reliability of NP research (Wang *et al*., 2022).

## 14. Conclusion

Network pharmacology has emerged as a valuable systems-level approach for understanding the complex, multitarget nature of drug action. By integrating bioactive compounds, molecular targets, pathways, diseases, molecular docking, ADME analysis, and experimental validation, it provides a systematic framework for modern drug discovery. Its integration with network ethnopharmacology is particularly useful for investigating natural products and traditional medicines. Recent advances in artificial intelligence, multiomics, structural biology, and drug repurposing are further expanding the potential of network-based approaches. However, limitations such as incomplete databases, prediction errors, lack of standardization, and limited experimental and clinical validation remain important challenges. Future progress will depend on improving data quality, developing reproducible workflows, and strengthening computational-experimental integration. Overall, network pharmacology offers a promising bridge between traditional medicinal knowledge, systems biology, and evidence-based drug discovery.

## Acknowledgements

The authors would like to acknowledge and thank RBVRR Women's College of Pharmacy, Barkatpura, Hyderabad, Telangana, India, for their continued support throughout this review.

## Availability of data and material

All data are provided within the manuscript.

## Consent for publication

All authors gave their full consent for publication and submission to this journal.

## Funding

The author stated that the work presented in this article received no associated funding.

## Ethics approval

Not applicable.

## Conflict of interest

The author declares no conflict of interest relevant to this article.

## References

**Barabási, A.L. and Oltvai, Z. N. (2004).** Network biology: understanding the cell's functional organization. Nat. Rev. Genet., **5**:101-113.

**Buriani, A.; Garcia-Bermejo, M. L.; Bosisio, E.; Xu, Q.; Li, H. and Dong, X. (2012).** Omic techniques in systems biology approaches to traditional Chinese medicine research: present and future. J. Ethnopharmacol., **140**:535-544.

**Chandran, U.; Mehendale, N.; Patil, S.; Chaguturu, R. and Patwardhan, B. (2017).** Network Pharmacology. In: Innovative Approaches in Drug Discovery, 127-164.

**Cho, D. Y.; Kim, Y.A. and Przytycka, T. M. (2012).** Chapter 5: network biology approach to complex diseases. PLoSComput. Biol., **8**:e1002820.

**Clark, D. E. and Grootenhuis, P. D. J. (2002).** Progress in computational methods for the prediction of ADMET properties. Curr. Opin. Drug Discov. Devel., **5**:382-390.

**Clark, D. E. and Pickett, S. D. (2000).** Computational methods for the prediction of "drug-likeness." Drug Discov. Today, **5**:49-58.

**Cui, G.; Li, M.; Guo, W.; Gao, M.; Zhu, Q. and Liao, J. (2025).** AI driven network pharmacology: Multi-scale mechanisms of traditional Chinese medicine from molecular to patient analysis. Comput. Struct. Biotechnol. J., **27**:5087-5104.

**Ellingson, S. R.; Smith, J. C. and Baudry, J. (2014).** Polypharmacology and supercomputer-based docking: opportunities and challenges. Mol. Simul., **40**:848-854.

**Fang, X.; Shao, L.; Zhang, H. and Wang, S. (2005).** CHMIS-C: A comprehensive herbal medicine information system for cancer. J. Med. Chem., **48**:1481-1488.

**Gu, J.; Chen, Y.; Li, S. and Li, Y. (2010).** Identification of responsive gene modules by network-based gene clustering and extending: Application to inflammation and angiogenesis. BMC Syst. Biol., **4**:47.

**Hacker, M.; Messer, W. S. and Bachmann, K. A. (2009).** Pharmacology: Principles and Practice. Elsevier/Academic Press.

**Hamosh, A.; Scott, A. F.; Amberger, J. S.; Bocchini, C. A. and McKusick, V. A. (2005).** Online mendelian inheritance in man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res., **33**:D514-D517.

**Harvey, A. L. (2008).** Natural products in drug discovery. Drug Discov. Today, **13**:894-901.

**Hopkins, A. L. (2007).** Network pharmacology. Nat. Biotechnol., **25**:1110-1111.

**Hopkins, A. L. (2008).** Network pharmacology: The next paradigm in drug discovery. Nat. Chem. Biol., **4**:682-690. doi: 10.1038/nchembio.118.

**Huang, Y. and Li, S. (2010).** Detection of characteristic sub pathway network for angiogenesis based on the comprehensive pathway network. BMC Bioinformatics, **11**.

**Kibble, M.; Saarinen, N.; Tang, J.; Wennerberg, K.; Mäkelä, S. and Aittokallio, T. (2015).** Network pharmacology applications to map the unexplored target space and therapeutic potential of natural products. Nat. Prod. Rep., **32**:1249-1266.

**Leung, E. L.; Cao, Z.W.; Jiang, Z. H.; Zhou, H. and Liu, L. (2013).** Network-based drug discovery by integrating systems biology and computational technologies. Brief. Bioinform., **14**:491-505.

**Li, L.; Mohammed, A. H.; Auda, N. A.; Alsallameh, S. M. S.; Albekairi, N. A.; Muhseen, Z. T. and Butch, C. J. (2023).** Network pharmacology, molecular docking, and molecular dynamics simulation analysis reveal insights into

the molecular mechanism of cordia myxa in the treatment of liver cancer. Biology, **13**(5):315.

**Li, S.; Wu, L. and Zhang, Z. (2006).** Constructing biological networks through combined literature mining and microarray analysis: A LMMA approach. Bioinformatics, **22**:2143-2150.

**Li, Y.; Li, R.; Ouyang, Z. and Li, S. (2015).** Herb network analysis for a famous TCM doctor's prescriptions on treatment of rheumatoid arthritis. Evid. Based Complement. Alternat. Med., **2015**:451319.

**Lu, L.; Li, Y. and Li, S. (2011).** Computational identification of potential microRNA network biomarkers for the progression stages of gastric cancer. Int. J. Data Min. Bioinform., **5**:519-531.

**Luo, F.; Gu, J.; Zhang, X.; Chen, L.; Cao, L. and Li, N. (2015).** Multiscale modeling of drug-induced effects of ReDuNing injection on human disease: from drug molecules to clinical symptoms of disease. Sci. Rep., **5**:10064.

**Merecz-Sadowska, A.; Sadowski, A.; Zieliñska-BliŸniewska, H.; Zajdel, K. and Zajdel, R. (2025).** Network pharmacology as a tool to investigate the antioxidant and anti-inflammatory potential of plant secondary metabolites: A review and perspectives. Int. J. Mol. Sci., **26**(14):6678.

**Noor, F.; Tahir ul Qamar, M.; Ashfaq, U. A.; Albutti, A.; Alwashmi, A. S. S. and Aljasir, M. A. (2022).** Network Pharmacology Approach for Medicinal Plants: Review and Assessment. Pharmaceuticals, **15**(5):572.

**Patwardhan, B. (2014).** Rediscovering drug discovery. Comb. Chem. High Throughput Screen., **17**:819.

**Patwardhan, B. and Chandran, U. (2015).** Network ethnopharmacology approaches for formulation discovery. Indian J. Tradit. Knowl., **14**:574-580.

**Patwardhan, B.; Mutalik, G. and Tillu, G. (2015).** Integrative Approaches for Health: Biomedical Research, Ayurveda and Yoga. Academic Press, Elsevier Inc.

**Pei, L.; Bao, Y.; Liu, S.; Zheng, J. and Chen, X. (2013).** Material basis of Chinese herbal formulas explored by combining pharmacokinetics with network pharmacology. PLoS One, **8**:e57414.

**Re, M. and Valentini, G. (2013).** Network-based drug ranking and repositioning with respect to DrugBank therapeutic categories. IEEE/ACM Trans. Comput. Biol. Bioinform., **10**:1359-1371.

**Saha, S. and Kunda, A. (2026).** Network pharmacology in drug discovery: concepts, methodology, applications and future perspectives. Int. J. Pharm. Sci., **4**(6):7044-7057.

**Van de Waterbeemd, H. and Gifford, E. (2003).** ADMET *in silico* modelling: towards prediction paradise? Nat. Rev. Drug Discov., **2**:192-204.

**Vithalkar, M. P.; Sandra, K. S.; Bharath, H. B.; Krishnaprasad, B.; Fayaz, S. M.; Sathyanarayana, B. and Nayak, Y. (2025).** Network pharmacology-driven therapeutic interventions for interstitial lung diseases using traditional medicines: A narrative review. Int. Immunopharmacol., **147**:113979.

**Wetzel, S.; Bon, R. S.; Kumar, K. and Waldmann, H. (2011).** Biology-oriented synthesis. Angew. Chem. Int. Ed., **50**:10800-10826.

**Wu, X.; Jiang, R.; Zhang, M. Q. and Li, S. (2008).** Network-based global inference of human disease genes. Mol. Syst. Biol., **4**:189.

**Xu, H.; Zhang, Y.; Lei, Y.; Gao, X.; Zhai, H. and Lin, N. (2014).** A systems biology-based approach to uncovering the molecular mechanisms underlying the effects of dragon's blood tablet in colitis, involving the integration of chemical analysis, ADME prediction, and network pharmacology. PLoS One, **9**:e101432.

**Yang, L.; Wang, H.; Zhu, Z.; Yang, Y.; Xiong, Y.; Cui, X. and Liu, Y. (2025).** Network Pharmacology-driven sustainability: AI and multiomics synergy for drug discovery in traditional Chinese medicine. Pharmaceuticals, **18**(7):1074.

**Yao, X.; Hao, H.; Li, Y. and Li, S. (2011).** Modularity-based credible prediction of disease genes and detection of disease subtypes on the phenotype-gene heterogeneous network. BMC Syst. Biol., **5**:79.

**Yildirim, M. A.; Goh, K.; Cusick, M. E.; Barabási, A. and Vidal, M. (2007).** Drug-target network. Nat. Biotechnol., **25**:1119-1126.

**Zahoránszky-Kõhalmi, G.; Siramshetty, V. B.; Kumar, P.; Gurumurthy, M.; Grillo, B.; Mathew, B.; Metaxatos, D.; Backus, M.; Mierzwa, T.; Simon, R.; Grishagin, I.; Brovold, L.; Mathé, E. A.; Hall, M. D.; Michael, S. G.; Godfrey, A. G.; Mestres, J.; Jensen, L. J. and Oprea, T. I. (2022).** A workflow of integrated resources to catalyze network pharmacology driven COVID-19 research. J. Chem. Inf. Model., **62**(3):718-729.

**Zhang, H. P.; Pan, J. B.; Zhang, C.; Ji, N.; Wang, H. and Ji, Z. L. (2014).** Network understanding of herb medicine *via* rapid identification of ingredient-target interactions. Sci. Rep., **4**:3719.

**Zhao, S. and Li, S. (2012).** A co-module approach for elucidating drug-disease associations and revealing their molecular basis. Bioinformatics, **28**:955-961.

**Zimmermann, G. R.; Lehár, J. and Keith, C. T. (2007).** Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discov. Today, **12**:34-42.

**Citation** **Fatima Umaira Saeed (2026). Network pharmacology in modern drug discovery: Principles, computational approaches, network ethnopharmacology and experimental validation. J. Phytonanotech. Pharmaceut. Sci., 6(3):84-92, http://dx.doi.org/10.54085/ap.2026.6.3.9.**