

# Network Pharmacology and Phytochemical Investigation of *Azadirachta Indica* Leaf

Avinash Manohar Bakle<sup>1</sup>, Umme Roman Shaikh<sup>2</sup>

<sup>1</sup>student, <sup>2</sup>Assistant Professor

<sup>1,2</sup>Department of pharmacy, Sayali charitable trust's college of pharmacy

## Abstract:

Because of its many therapeutic uses, *Azadirachta indica*, or neem, is a medicinally significant plant that is frequently utilized in conventional healthcare systems. Using a network pharmacology technique, the current study was carried out to assess the phytochemical components and pharmacological importance of neem leaves. Fresh neem leaves were gathered, shade-dried, ground into a powder, and then extracted using the maceration process with either methanol or ethanol. A preliminary phytochemical screening was performed on the produced extracts in order to identify significant secondary metabolites. Alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids were all found, according to qualitative examination. These phytochemicals, which are well-known for their biological properties, greatly enhance neem's therapeutic potential in both conventional and contemporary uses across the globe. Quercetin, rutin, hyperoside, quercitrin, nimbolide, azadirachtin, and nimbaflavone are among the significant phytoconstituents found in neem leaves that were chosen for additional pharmacological analysis. These compounds' pharmacokinetic characteristics and drug-likeness were examined utilizing common scientific databases like PubChem, IMPPAT, and TCMSP. The results showed that neem phytochemicals had potent anti-inflammatory, immunomodulatory, anticancer, antioxidant, and antibacterial properties. These substances interact with several biological targets and disease-related signaling pathways, suggesting a multi-target mechanism of action, according to network pharmacology analysis. Quercetin and rutin showed very encouraging drug-likeness ratings and pharmacological potential among the assessed phytoconstituents. The study demonstrates the value of network pharmacology in herbal drug development and therapeutic research while providing scientific validation for the traditional medicinal significance of neem.

## 1. INTRODUCTION

### 1.1 Introduction to *Azadirachta indica* (neem):

Neem, or *Azadirachta indica*, is an evergreen tree of the Meliaceae family that grows quickly. It is a native of the Indian subcontinent and is found in many tropical and subtropical regions of the world, including Bangladesh, Pakistan, and India. Neem is suitable for arid and semi-arid regions because it can adapt well to extreme climatic circumstances, such as dry climates, nutrient-poor soils, and high temperatures. The tree has a wide spreading crown and often reaches a height of 15 to 30 meters. Pinnate green foliage, fragrant white flowers, and smooth, olive-like fruits are some of its botanical characteristics. Neem has emerged as a key species for environmental sustainability, afforestation programs, and rural development initiatives in many developing nations worldwide due to its remarkable adaptability and ecological resilience.

For many decades, neem has played a vital role in traditional medical systems, particularly Ayurveda. The plant's leaves, bark, seeds, roots, and flowers are among the various parts that are used for medicinal purposes. Neem leaves are useful for healing wounds, skin conditions, and infections because of their antibacterial, antifungal, anti-inflammatory, and antioxidant qualities. Traditionally, the bark has been used to make "datun," a chewing stick that helps keep teeth and gums healthy and encourages good dental hygiene. Many dermatological disorders, including lice infestations and dandruff, are treated with neem

seed oil. Neem is widely used in herbal formulations, cosmetics, and healthcare items because of its extensive therapeutic value. Globally, scientific study into contemporary therapeutic usage is still motivated by its traditional applications.

Neem has significant use in agriculture and environmental management in addition to its therapeutic value. Because it decreases nitrogen loss from fertilizers and increases crop nutrient efficiency, neem-coated urea is widely employed as a nitrification inhibitor. Additionally, this procedure reduces environmental contamination brought on by runoff and nitrogen leaching. Because it is effective against a variety of agricultural pests, neem serves as a natural biopesticide and is particularly valuable in integrated pest control systems. Neem-based products are relatively safer for people, animals, and the environment than many synthetic pesticides. Important bioactive substances with hepatoprotective, immunomodulatory, anticancer, and antioxidant properties include nimbolide and limonoids, according to scientific research. These substances may effectively stop the growth of tumors and cause malignant cells to undergo apoptosis.

Additionally, neem makes a substantial contribution to environmental restoration and ecological conservation efforts. The tree is very advantageous for land reclamation and soil rehabilitation initiatives since it can flourish in deteriorated, salty, and nutrient-deficient soils. In both urban and rural areas, its thick evergreen canopy improves microclimatic conditions, reduces ambient temperatures, and offers shade. By providing habitat and cover for insects, birds, and other creatures, neem plantations promote biodiversity. Neem is also known for its capacity to sequester carbon, which lowers atmospheric carbon dioxide levels and aids in efforts to mitigate climate change. Neem is frequently planted in urban forestry initiatives, roadside plantations, shelterbelts, and windbreak systems due to these ecological advantages, especially in tropical and semi-arid countries dealing with environmental stress and desertification issues.

### 1.1.2 Taxonomical Classification of Neem:

Sr.no	Taxonomic rank	Classification
1.	Kingdom	Plantae(plant)
2.	Subkingdom	Tracheobionta
3.	Superdivision	Spermatyophyta (seed plant)
4.	Division	Magnoliophyta(flowering plant)
5.	Class	Magnoliopsida
6.	Subclass	Rosidae
7.	Family	Meliaceae
8.	Genus	Azadirachta
9.	Species	A.indica

### 1.1.3 Geographical Distribution:

*Azadirachta indica*, commonly called neem, originated in the Indian subcontinent and is now widely distributed throughout tropical and subtropical regions of the world. Due to its strong adaptability, the tree has become naturalized in parts of Africa, Southeast Asia, Central America, and South America. Neem grows well in hot climates with low rainfall and prefers well-drained soils. Its remarkable tolerance to drought and poor soil conditions has made it valuable in dryland afforestation, environmental conservation, and traditional healthcare systems across countries.

### 1.1.4 Morphological Features:

- Structure:

Neem is a medium to large evergreen tree that can attain a height of approximately 15–30 meters. It possesses a spreading canopy with dense foliage and a rough greyish-brown bark marked by deep fissures.

- Leaves:

Native to the Indian subcontinent, the Neem tree (*Azadirachta indica*) belongs to the Meliaceae family

and is recognized by its dark green, pinnate compound leaves with serrated, asymmetrical leaflets. These leaves possess a signature bitter taste due to bioactive compounds like Azadirachtin and nimbin, which provide antimicrobial, anti-inflammatory, and antioxidant benefits used to treat skin conditions, infections, and dental problems. Beyond medicine, neem serves as an eco-friendly tool in agriculture and domestic life, where leaf extracts act as natural pesticides and dried leaves protect stored grains from pests.

- The tree produces small, white, aromatic flowers that grow in hanging clusters. These flowers have a mild honey-like fragrance.

- **Fruit:**

Neem fruits are smooth, oval-shaped drupes that turn yellowish-green on ripening. Each fruit generally contains a single seed rich in oil content.

- **Roots:**

The plant develops a strong taproot system capable of penetrating deep into the soil, allowing the tree to survive under drought and nutrient-deficient conditions.

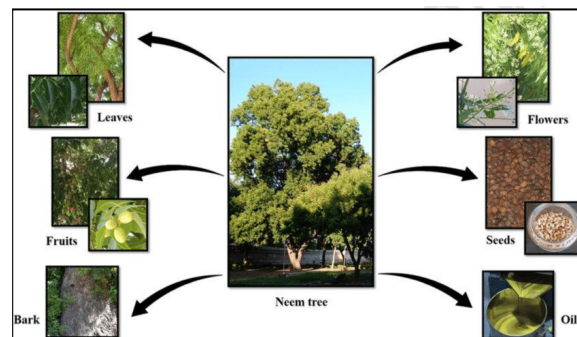


Fig 1.1: Morphological structure of Neem plant

### 1.1.5 Medicinal Uses of Neem:

- **Skin care:** Neem is extensively used for treating acne, eczema, fungal infections, and other skin disorders because of its antibacterial and antifungal activities.
- **Dental Health:** Neem bark and twigs have traditionally been used as natural toothbrushes or “datun” to maintain oral hygiene and reduce gum diseases and dental caries.
- **Wound Healing:** Its antiseptic properties help cleanse wounds and support faster regeneration of damaged tissues.
- **Hair Care:** Neem oil and leaf extracts are commonly applied to control dandruff and eliminate head lice.
- **Internal Health:** Traditionally, neem has been used to lower fever, regulate blood glucose levels, and remove intestinal worms.
- **Immune Support:** The presence of antioxidant compounds in neem helps detoxify the body and strengthen immune function.

### 1.1.6 Pharmacological Activities:

- **Antimicrobial Activity:** Neem exhibits inhibitory effects against a wide range of bacteria, fungi, and viruses by interfering with microbial growth and replication.
- **Anti-inflammatory Activity:** Bioactive compounds present in neem reduce inflammation and pain by suppressing inflammatory pathways and enzymes such as COX and LOX.
- **Antioxidant Activity:** Neem contains flavonoids and polyphenolic compounds, including quercetin, which help neutralize harmful free radicals and protect cells from oxidative stress.
- **Anticancer Activity:** Certain constituents, particularly nimbolide, have shown potential in research studies for inducing programmed cell death in cancer cells and restricting tumor progression.

- Hypoglycemic Activity: Neem may contribute to blood sugar regulation by improving insulin action and enhancing glucose utilization in the body.
- Immunomodulatory Activity: It supports immune defense by stimulating the activity of immune cells such as macrophages and lymphocytes.

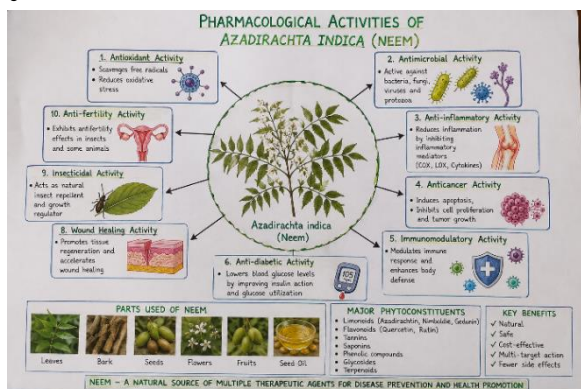


Fig 1.2: Pharmacological properties of neem plant

### 1.1.7 Other Uses:

- Agricultural Applications: Neem is widely used as a natural pesticide and organic manure. Neem-coated urea improves nitrogen efficiency by slowing nutrient loss and reducing environmental contamination.
- Environmental Applications: The tree plays an important role in reforestation, soil improvement, and control of desertification. Its canopy also helps reduce air pollution and provides shade in urban areas.
- Domestic Uses: Dried neem leaves are traditionally stored with grains to protect them from insect infestation, and neem smoke or oil is often used as a mosquito repellent.
- Industrial Uses: Neem is utilized in the manufacture of soaps, shampoos, cosmetics, and personal care products. Its wood is durable and resistant to termites, making it useful for furniture and agricultural implements.

## 1.2 Introduction of Neem leaf

*Azadirachta indica*, commonly known as neem, is a medicinally important evergreen tree belonging to the family Meliaceae. It is native to the Indian subcontinent and is widely distributed in tropical and subtropical regions. Neem has been used for centuries in Ayurveda, Unani, Siddha, and traditional medicine systems because of its broad therapeutic properties. Among all parts of the neem plant, the leaves are the most widely used for medicinal purposes. Neem leaves are rich in bioactive phytochemicals such as flavonoids, alkaloids, tannins, terpenoids, glycosides, phenolic compounds, nimbin, quercetin, and azadirachtin. These compounds contribute to various pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, antifungal, antidiabetic, immunomodulatory, and anticancer effects. Neem leaves are traditionally used for treating skin disorders, wounds, fever, infections, dental problems, and digestive disorders. Due to their medicinal importance and safety profile, neem leaves have gained significant attention in modern pharmaceutical and herbal research.

### 1.2.1 Botanical Description of Neem Leaf

Sr.no	Parameter	Description
1.	Botanical name	<i>Azadirachta indica</i> A. Juss.
2.	Family	Meliaceae
3.	Leaf type	Compound imparipinnate leaf
4.	Color	Dark green
5.	Shape	Lanceolate

6.	Surface	Smooth (glabrous)
7.	Odour	Characteristic
8.	Taste	Bitter
9.	Texture	Thin and papery

### 1.2.2 Morphological Characteristics of Neem Leaf

- Color: Neem leaves are dark green in color and remain evergreen throughout most of the year.
- Shape: The leaflets are lanceolate or elongated with pointed tips.
- Margin: Leaf margins are serrated or toothed.
- Venation: Neem leaves show pinnate venation.
- Odor: Leaves possess a characteristic medicinal odor.
- Taste: Neem leaves are highly bitter because of limonoids and related compounds.

### 1.2.3 Chemical Constituents of Neem Leaves

Neem leaves contain several important phytochemicals including: 1)Alkaloids, 2)Flavonoids, 3)Tannins, 4)Saponins, 5)Glycosides, 6)Phenolic compounds 7)Terpenoids, 8) Quercetin, 9) Nimbin, 10)Nimbolide, 11) Azadirachtin, 12)Rutin

### 1.2.4 Medicinal Uses of Neem Leaves

- Skin Disorders: Used in acne, eczema, fungal infections, and wounds.
- Dental Care: Neem twigs and leaves are used for oral hygiene and gum protection.
- Fever and Infections: Traditionally used to reduce fever and treat infections.
- Hair Care: Neem leaf extracts help control dandruff and lice.
- Blood Purification: Used traditionally as a detoxifying and blood-purifying agent.
- Diabetes Management: Helps in controlling blood sugar levels.

### 1.2.5 Other Important Uses of Neem Leaves

- Agricultural Use: Neem leaves are used as natural pesticides and insect repellents.
- Grain Protection: Dried leaves are stored with grains to prevent insect infestation.
- Cosmetic Industry: Neem extracts are used in soaps, creams, shampoos, and herbal products.
- Environmental Importance: Neem contributes to environmental protection through soil improvement and pest control.

### 1.2.6 Advantages of Neem Leaves

- Natural and eco-friendly
- Cost-effective medicinal source
- Easily available
- Possess multiple therapeutic activities
- Lower side effects compared to synthetic drugs



Fig 1.3: Morphological structure of Neem leaf

### **1.3 Introduction of Network Pharmacology**

Network pharmacology is a contemporary method of drug discovery and development that uses a network-based system to investigate how medications, genes, proteins, targets, and diseases interact. Network pharmacology adheres to the "multiple drugs–multiple targets" paradigm, which allows various substances to operate on several biological pathways at once, in contrast to the conventional "one drug–one target" approach. To comprehend therapeutic mechanisms and forecast medication responses, this approach integrates pharmacology, bioinformatics, systems biology, and computer science. Because medicinal plants contain many active phytochemicals that combine to create therapeutic effects, network pharmacology is very helpful in herbal medicine research. It is now a crucial scientific tool for researching medicinal plants like *Azadirachta indica* and successfully comprehending their intricate pharmacological actions.

#### **1.3.1 Concept of Network pharmacology:**

The knowledge that medications interact with several biological targets rather than just one is the foundation of the idea of network pharmacology. Using a systems-based approach, it investigates the intricate connections between substances, proteins, genes, processes, and illnesses. Network pharmacology explains how various substances interact to elicit therapeutic responses by integrating pharmacology, bioinformatics, systems biology, and computer research. This idea is particularly crucial for research on herbal medicine since medicinal plants have many bioactive components that can affect several signaling pathways at once. Network pharmacology aids in the identification of active phytochemicals, the prediction of target proteins, the analysis of disease-related pathways, and the comprehension of the overall mechanism of action in plants such as neem. As a result, it offers a thorough comprehension of drug action and illness treatment at the molecular and systemic levels.

#### **1.3.2 Steps in network pharmacology:**

- Identification of active compounds
- Target prediction
- Disease target collection
- Common target identification
- Network construction
- Pathway enrichment analysis
- Molecular docking
- Data interpretation and validation

#### **1.3.3 Applications of Network Pharmacology**

1. Drug discovery and development
2. Herbal medicine research
3. Identification of drug targets
4. Disease mechanism analysis
5. Multi-target therapy analysis
6. Biomarker identification
7. Personalized medicine
8. Toxicity and safety prediction
9. Pathway analysis
10. Study of drug–drug interactions

#### **1.3.4 Importance of Medicinal Plants**

1. Source of natural medicines
2. Used in treatment of various diseases

3. Fewer side effects compared to synthetic drugs
4. Cost-effective and easily available
5. Rich in bioactive phytochemicals
6. Used in traditional medicine systems
7. Important in drug discovery and research
8. Possess antimicrobial, antioxidant, and anti-inflammatory properties
9. Support healthcare in rural areas
10. Economically valuable for pharmaceutical industries

### **1.3.5 Network Pharmacology Helps To**

1. Identify active compounds
2. Predict drug targets
3. Understand disease mechanisms
4. Analyze biological pathways
5. Study multi-target drug action
6. Discover new drugs
7. Evaluate herbal medicines
8. Reduce drug toxicity
9. Improve therapeutic effectiveness
10. Support personalized medicine

### **1.3.6 Advantages of Network Pharmacology**

1. Studies multiple targets simultaneously
2. Explains complex disease mechanisms
3. Supports herbal medicine research
4. Improves drug discovery process
5. Reduces time and cost of research
6. Helps identify new therapeutic targets
7. Predicts drug interactions and toxicity
8. Provides system-level understanding
9. Supports personalized medicine
10. Enhances treatment effectiveness

### **1.3.7 Limitations of Network Pharmacology**

1. Depends on database accuracy
2. Requires advanced bioinformatics tools
3. Complex data analysis
4. Limited experimental validation
5. Prediction results may not always be accurate
6. High computational requirements
7. Difficult to interpret large networks
8. Incomplete biological information in databases
9. Time-consuming validation process
10. Requires multidisciplinary knowledge

## **1.4 Introduction software used in network pharmacology**

### **1.4.1 Dr. Duke's Phytochemical and Ethnobotanical Databases:**

Network pharmacology research frequently use Dr. Duke's Phytochemical and Ethnobotanical Databases to gather data on therapeutic plants and their phytochemical components. The program aids scientists in

locating bioactive substances found in plants and comprehending their medicinal qualities. It offers information on biological processes, plant-compound interactions, and conventional medical applications. Dr. Duke's database is frequently utilized in network pharmacology to promote herbal medicine research, screen for active phytochemicals, and choose possible compounds for target prediction. It is a valuable source of phytochemical information for researching the workings of medicinal plants and how they are used to treat illness.

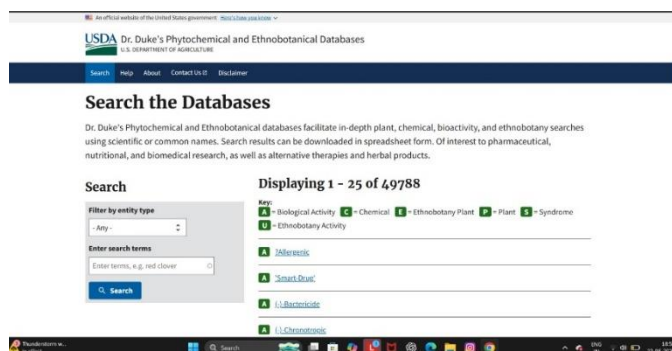


Fig 1.4: Dr. Duke's phytochemical software homepage

## 1.4.2 IMPPAT:

In network pharmacology research, IMPPAT is a crucial database for gathering data on Indian medicinal plants, phytochemicals, and their therapeutic properties. It offers information on the chemical characteristics, target proteins, and pharmacological effects of bioactive chemicals found in medicinal plants. Research on herbal drugs, phytochemical screening, target prediction, and pathway analysis all make extensive use of IMPPAT. By offering scientifically verified data that is helpful in network pharmacology and drug discovery research, the database promotes the study of plant-based medications.

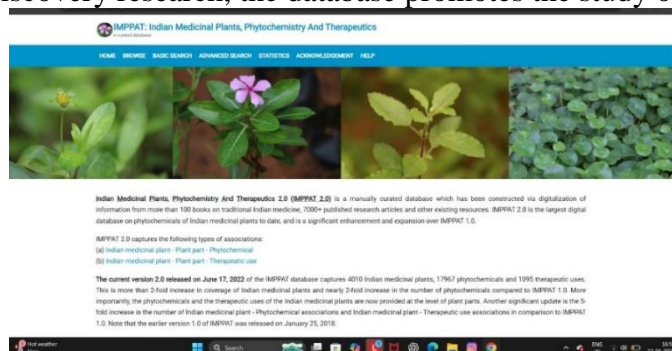


Fig 1.5: IMPPAT software homepage

## 1.4.3 MolSoft:

MolSoft is a computational program that is frequently used in drug development, molecular modeling, and network pharmacology. By using molecular docking and virtual screening approaches, it helps researchers investigate interactions between bioactive chemicals and target proteins. Important tools for predicting drug-likeness, pharmacokinetic characteristics, and chemical compound binding affinity are provided by the software. By examining compound-target interactions and assessing biological activity, MolSoft assists in the identification of possible therapeutic compounds. It facilitates comprehension of pharmacological mechanisms and pathway connections in network pharmacology, particularly in pharmaceutical and medicinal plant research. The program is a crucial tool in contemporary pharmacological and biological research since it can shorten research times and increase computational drug design accuracy.

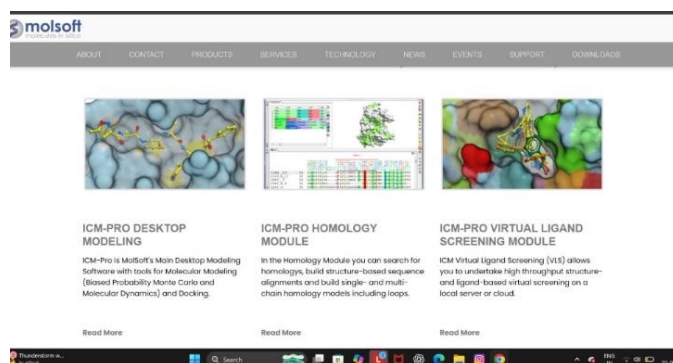


Fig 1.6: MolSoft software homepage

### 1.5.5 ProTox 3.0

Network pharmacology and drug discovery studies use ProTox 3.0, an online toxicity prediction engine, to assess the safety profile of bioactive chemicals. Using computational techniques, it assists researchers in predicting toxicological characteristics like hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. Before conducting experiments, ProTox 3.0 is frequently used to evaluate the toxicity of phytochemicals and potential drugs. By detecting possible harmful effects and lowering the possibility of negative reactions in pharmaceutical and medical research, it promotes safer drug development.

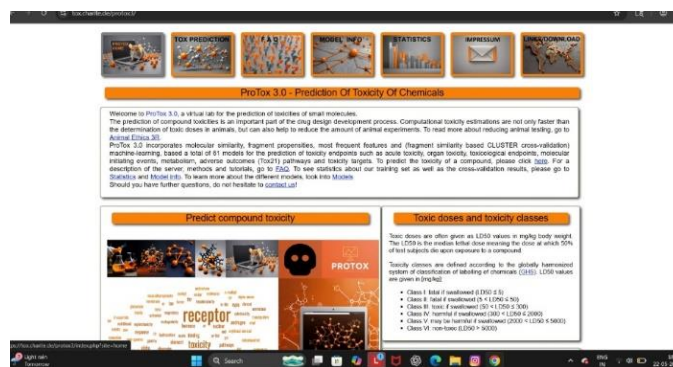


Fig1.7: ProTox 3.0 software homepage

### 1.4.5 SwissADME:

SwissADME is an online tool used in drug development and network pharmacology research to assess the ADME characteristics of bioactive substances. The acronym representing absorption, distribution, metabolism, and excretion is ADME. Researchers can use the software to forecast a compound's physicochemical qualities, blood-brain barrier permeability, gastrointestinal absorption, bioavailability, and drug-likeness. SwissADME is frequently used in network pharmacology to screen phytochemicals and choose possible drug candidates with appropriate pharmacokinetic characteristics for additional therapeutic study.

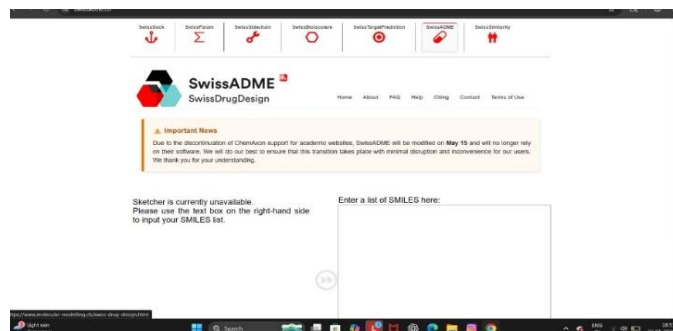


Fig 1.8: SwissADME software home page

## **2.LITERATURE REVIEW**

### **2.1 Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach**

In ethnomedicine, plant parts and compounds are used traditionally to treat different diseases. Neem (*Azadirachta indica* A. Juss) is the most versatile and useful medicinal plant ever found. Its every part is rich in bioactive compounds, which have traditionally been used to treat different ailments including infectious diseases. Bioactive compounds such as nimbolide, azadirachtin, and gedunin of neem are reported to have a tremendous ability to regulate numerous biological processes in vitro and in vivo. The present review article aims to explore the importance of neem extracts and bioactive compounds in the regulation of different biological pathways. We have reviewed research articles up to March 2020 on the role of neem in antioxidant, anti-inflammatory, antiangiogenic, immunomodulatory, and apoptotic activities. Studies on the concerned fields demonstrate that the bioactive compounds and extracts of neem have a regulatory effect on several biological mechanisms. It has been unveiled that extensive research is carried out on limonoids such as nimbolide and azadirachtin. It is evidenced by different studies that neem extracts are the potential to scavenge free radicals and reduce ROS-mediated damage to cells. Neem can be used to normalize lipid peroxidation and minimize ROS-mediated cell death. Besides, neem extracts can significantly reduce the release of proinflammatory cytokines and elevate the count of CD4 + and CD8 + T-cells. This review indicates the pivotal roles of *A. indica* in the regulation of different biological pathways. However, future investigations on other bioactive compounds of neem may reveal different therapeutic potentials.(1)

### **2.2 Limonoids from neem (*Azadirachta indica* A. Juss.) are potential anticancer drug candidates**

Neem (*Azadirachta indica* A. Juss.), a versatile evergreen tree recognized for its ethnopharmacological value, is a rich source of limonoids of the triterpenoid class, endowed with potent medicinal properties. Extracts of neem have been documented to display anticancer effects in diverse malignant cell lines as well as in preclinical animal models that has largely been attributed to the constituent limonoids. Of late, neem limonoids have become the cynosure of research attention as potential candidate agents for cancer prevention and therapy. Among the various limonoids found in neem, azadirachtin, epoxyazadiradione, gedunin, and nimbolide, have been extensively investigated for anticancer activity. Azadirachtin, a potent biodegradable pesticide, exhibits profound antiproliferative effects by preventing mitotic spindle formation and cell division. The antiproliferative activity of gedunin has been demonstrated to be mediated primarily via inhibition of heat shock protein90 and its client proteins. Epoxyazadiradione inhibits pro-inflammatory and kinase-driven signaling pathways to block tumorigenesis. Nimbolide, the most potent cytotoxic neem limonoid, inhibits the growth of cancer cells by regulating the phosphorylation of keystone kinases that drive oncogenic signaling besides modulating the epigenome. There is overwhelming evidence to indicate that neem limonoids exert anticancer effects by preventing the acquisition of hallmark traits of cancer, such as cell proliferation, apoptosis evasion, inflammation, invasion, angiogenesis, and drug resistance. Neem limonoids are value additions to the armamentarium of natural compounds that target aberrant oncogenic signaling to inhibit cancer development and progression.(2)

### **2.3 Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment**

Neem (*Azadirachta indica*) is a member of the Meliaceae family and its role as health-promoting effect is attributed because it is rich source of antioxidant. It has been widely used in Chinese, Ayurvedic, and Unani medicines worldwide especially in Indian Subcontinent in the treatment and prevention of various diseases. Earlier finding confirmed that neem and its constituents play role in the scavenging of free radical generation and prevention of disease pathogenesis. The studies based on animal model established that neem and its chief constituents play pivotal role in anticancer management through the modulation of various molecular pathways including p53, pTEN, NF- $\kappa$ B, PI3K/Akt, Bcl-2, and VEGF. It is considered

as safe medicinal plants and modulates the numerous biological processes without any adverse effect. In this review, I summarize the role of *Azadirachta indica* in the prevention and treatment of diseases via the regulation of various biological and physiological pathways. (3)

## 2.4 Neem (*Azadirachta indica*): An indian traditional panacea with modern molecular basis

Neem (*Azadirachta indica*): An indian traditional panacea with modern molecular basis

*Azadirachta indica* (Neem) is a medicinal plant widely used in traditional medicine for the treatment of various acute and chronic diseases. Different parts of the plant, including leaves, flowers, bark, and seeds, possess important therapeutic properties such as antimicrobial, antibacterial, antiviral, antimalarial, anti-inflammatory, and insecticidal activities. Numerous scientific studies have identified more than 300 bioactive constituents in neem, including limonoids such as nimbolide, azadirachtin, and gedunin, which contribute to its pharmacological effects. These phytoconstituents act by modulating multiple cellular signaling pathways and biological targets. Network pharmacology studies have further demonstrated the multi-target therapeutic potential of neem compounds in disease management. The wide range of medicinal activities of neem highlights its significance as a valuable natural source for modern drug discovery and herbal medicine research. (4)

## 3. AIM AND OBJECTIVES

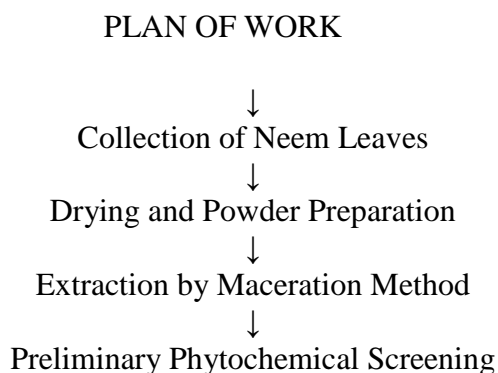
### 3.1 Aim:

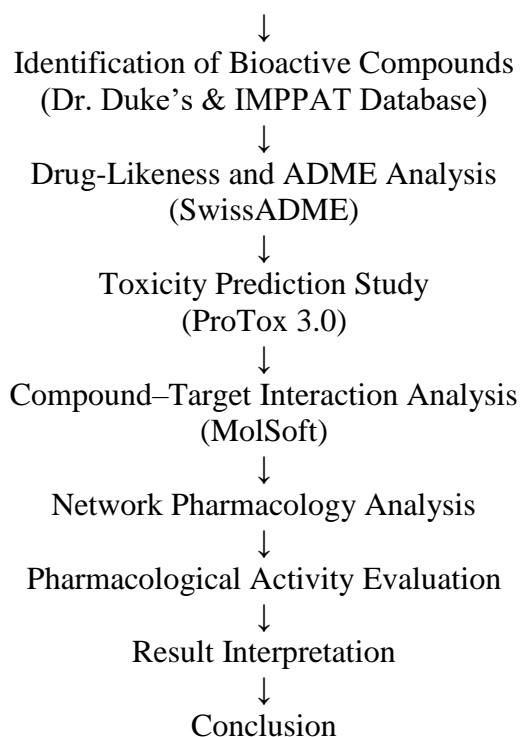
This study assesses the therapeutic qualities of neem leaves (*Azadirachta indica*) using a network pharmacology approach. In order to identify the multi-pathway biological mechanisms underlying the plant's therapeutic effects, the study attempts to map the intricate interactions between the plant's bioactive chemicals and their particular molecular targets. The study aims to establish a contemporary scientific basis for the conventional multi-target applications of neem in herbal medicine by determining these chemical-to-protein connections.

### 3.2 Objectives:

- To determine the main phytochemical components found in *Azadirachta indica* leaves.
- To assess the pharmacokinetic characteristics and drug-likeness of neem phytoconstituents using SwissADME.
- To use ProTox 3.0 to forecast the toxicity profiles of neem bioactive chemicals.
- To use network pharmacology techniques to examine compound–target interactions.
- To research neem compounds' pharmacological activity, including their anti-inflammatory, antioxidant, antibacterial, and anticancer qualities.
- To comprehend neem phytoconstituents' multi-target therapeutic processes.
- To offer scientific support for the conventional therapeutic applications of neem leaves.

## 4. PLAN OF WORK





## 5. METHODOLOGY

### 5.1 Authentication of Neem Plant Leaves

The plant material that was gathered was confirmed to be *Azadirachta indica* leaves, which are members of the Meliaceae family. Macroscopic and morphological traits such leaf shape, arrangement, border, color, and odor were used for authentication. A certified botanist/taxonomist from the Department of Botany identified and verified the leaves. A voucher specimen was created and kept in the institution's herbarium for further use.

#### Macroscopic Features of Neem Leaves

- Shade: green
- Odor: A distinctively bitter smell • Bitter flavor
- Type of Leaf: Compound imparipinnate leaves
- Lanceolate leaflets with serrated edges • Configuration: Change • Texture: a smooth surface with a sharp tip

### 5.2 Collection and Preparation of Plant Material:

To remove dirt and contaminants, fresh neem leaves were collected from healthy plants and cleansed with distilled water. To preserve heat-sensitive phytochemicals, the leaves were subsequently shade-dried at room temperature for around a week. The leaves were stored in sealed containers for additional extraction research after being completely dried and processed into a coarse powder using a mechanical grinder.



Fig 5.1: collection of neem leaf

**5.3 Preparation of Neem Leaf Extract:** Ethanol or methanol was used to extract 50–100 g of powdered neem leaves. Because of their efficiency in extracting bioactive phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids, these solvents were chosen.



Fig 5.2: filtration

#### 5.4 Maceration Method:

The maceration procedure involved soaking dried *Azadirachta indica* neem leaf powder in ethanol or methanol for approximately 72 hours at room temperature in a closed container. To improve the extraction of phytochemical components from the plant material into the solvent, the mixture was periodically agitated. Following the extraction phase, the mixture was filtered to get rid of the solid residue using Whatman filter paper or muslin cloth. A crude neem leaf extract high in bioactive components was obtained by concentrating the collected filtrate by evaporating the solvent at low temperature or under decreased pressure. In order to do additional phytochemical research, the extract was ultimately kept in sealed containers.

#### 5.5 Preliminary Phytochemical Screening:

The neem leaf extract was subjected to preliminary phytochemical screening for identification of different phytoconstituents.

➤ **Phytochemical test performs :**

Sr.no	Phytochemical	Test performed
1.	Alkaloids	Dragendorff's Test
2.	Flavonoids	Alkaline Reagent Test
3.	Tannins	Ferric Chloride Test
4.	Saponins	Foam Test
5.	Glycosides	Keller–Killiani Test
6.	Phenol	Ferric Chloride Test
7.	Terpenoids	Salkowski Test

➤ **Test procedure:**

Sr.no	Phytochemical Constituent	Method Used	Methodology	Observation	Result
5.5.1	Alkaloids	Dragendorff's Test	The neem leaf extract was dissolved in dilute hydrochloric acid and filtered. A few drops of Dragendorff's reagent were added to the filtrate.	Formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.	Alkaloids were present in the neem leaf extract.

5.5.2	Flavonoids	Alkaline Reagent Test	The extract was treated with sodium hydroxide solution followed by dilute hydrochloric acid	Development of yellow coloration that disappeared after acid addition confirmed flavonoids.	Flavonoids were present in the neem leaf extract.
5.5.3	Tannins	Ferric Chloride Test	Ferric chloride solution was added to the extract.	Blue-black or green coloration indicated the presence of tannins.	Tannins were present in the neem leaf extract.
5.5.4	Saponins	Foam Test	The extract was mixed with distilled water and shaken vigorously.	Persistent froth formation confirmed the presence of saponins.	Saponins were present in the neem leaf extract.
5.5.5	Glycosides	Keller–Killiani Test	The extract was treated with glacial acetic acid containing ferric chloride followed by concentrated sulfuric acid.	Formation of a brown ring at the interface confirmed glycosides.	Glycosides were present in the neem leaf extract.
5.5.6	Phenols	Ferric Chloride Test	Ferric chloride solution was added to the extract	Deep bluish-green coloration confirmed phenolic compounds.	Phenolic compounds were present in the neem leaf extract.
5.5.7	Terpenoids	Salkowski Test	The extract was mixed with chloroform and concentrated sulfuric acid.	Formation of a reddish-brown interface confirmed terpenoids.	Terpenoids were present in the neem leaf extract.

## 6. RESULTS AND DISCUSSION

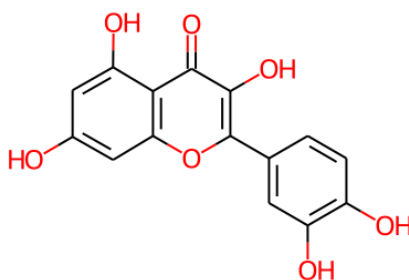
### 6.1 Phytochemical Screening Results:

Numerous significant secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids, were found in neem leaf extract by phytochemical screening. The wide range of neem's medicinal properties are caused by these phytoconstituents. By scavenging free radicals, flavonoids and phenolic substances greatly enhance antioxidant activity. While glycosides and saponins may have immunomodulatory effects, alkaloids and terpenoids have antibacterial and anti-inflammatory properties.

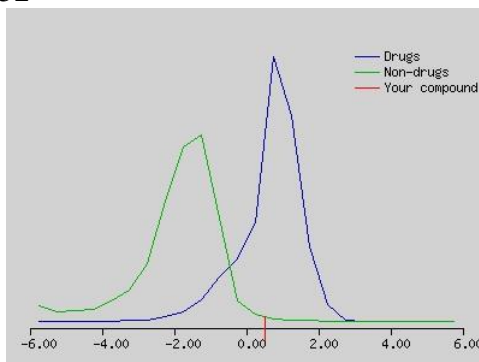
### 6.2 Phytoconstituent Analysis:

Sr. no	Chemical name	Activity count	Plant part	Molecular weight(g/mol)	HBA	HBD	Drug likeness score
1.	Quercetin	176	Leaf	302.04	7	5	0.52
2.	Rutin	87	Leaf	610.15(>500)	16(>10)	10(>5)	0.91
3.	Azadirachtan	0	Leaf	600.26(>500)	11(>10)	2	-0.07
4.	Hyperoside	30	Leaf	464.1	12(>10)	8(>5)	0.68
5.	Nimbolide	5	Leaf	466.5	7	0	-0.3
6.	Quercitrin	87	Leaf	448.4	11(>10)	7(>5)	0.82
7.	Nimbaflavone	0	Leaf	422.21	5	2	0.84

#### 1. Molecular structure of Quercetin:

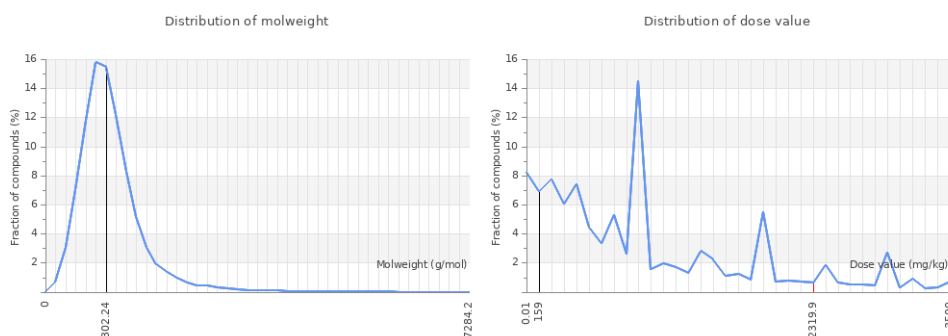




- Drug likeness of Quercetin:0.52



➤ Toxicity prediction of Quercetin:

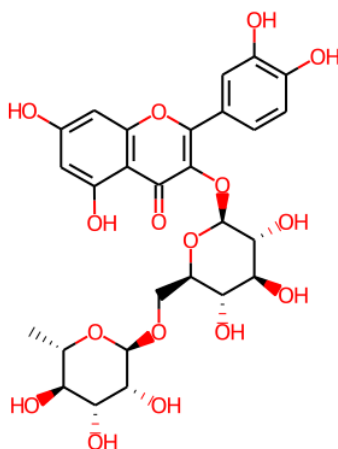
Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.69
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.89
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.62
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.83
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Inactive	0.99
Toxicity end points	<u>Carcinogenicity</u>	carcino	Active	0.68
Toxicity end points	<u>Immunotoxicity</u>	immuno	Inactive	0.87
Toxicity end points	<u>Mutagenicity</u>	mutagen	Active	0.51
Toxicity end points	<u>Cytotoxicity</u>	cyto	Inactive	0.99



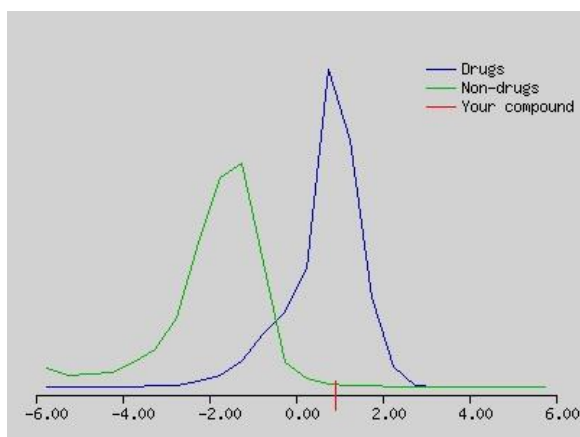
	Value of input compound
	Mean value of dataset

Predicted LD50: 159mg/kg of Quercetin

2. Molecular structure of Rutin:

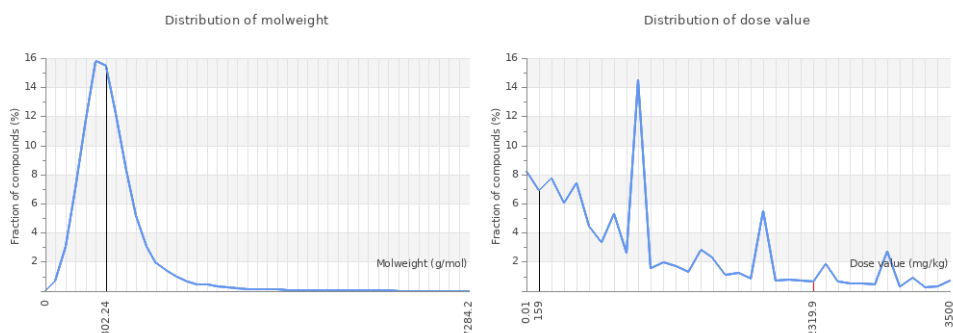


- Drug-likeness model score for Rutin: 0.91



➤ Toxicity prediction of Rutin:

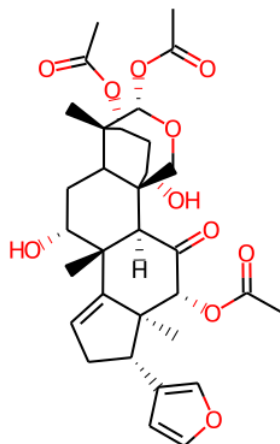
Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.80
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.89
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.77
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.63
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Inactive	0.98
Toxicity end points	<u>Carcinogenicity</u>	carcino	Inactive	0.91
Toxicity end points	<u>Immunotoxicity</u>	immuno	Active	0.98
Toxicity end points	<u>Mutagenicity</u>	mutagen	Inactive	0.88
Toxicity end points	<u>Cytotoxicity</u>	cyto	Inactive	0.64
Toxicity end points	<u>BBB-barrier</u>	bbb	Inactive	0.75



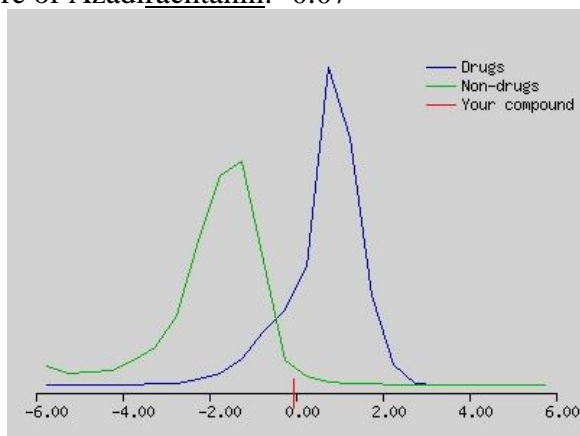
Value of input compound  
 Mean value of dataset

Predicted LD50: 5000mg/kg of Rutin

3. Molecular structure of Azadirachtanin:

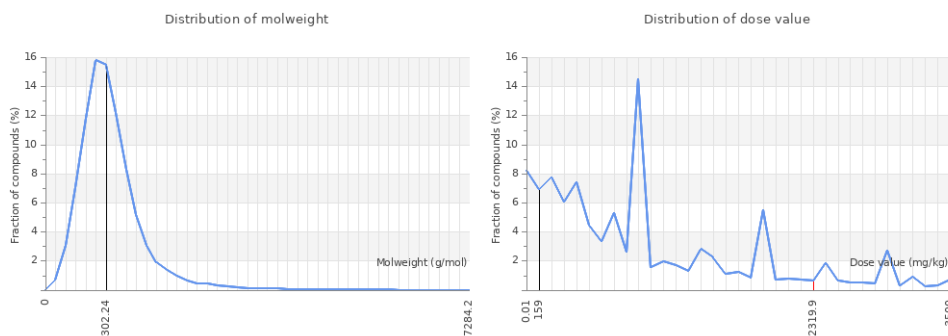


- Drug-likeness model score of Azadirachtanin: -0.07



➤ Toxicity prediction of Azadirachtanin:

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.91
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.85
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.58
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.76
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Inactive	0.6
Toxicity end points	<u>Carcinogenicity</u>	carcino	Inactive	0.60
Toxicity end points	<u>Immunotoxicity</u>	immuno	Active	0.98
Toxicity end points	<u>Mutagenicity</u>	mutagen	Inactive	0.73
Toxicity end points	<u>Cytotoxicity</u>	cyto	Inactive	0.57
Toxicity end points	<u>BBB-barrier</u>	bbb	Active	0.53
Toxicity end points	<u>Ecotoxicity</u>	eco	Inactive	0.68

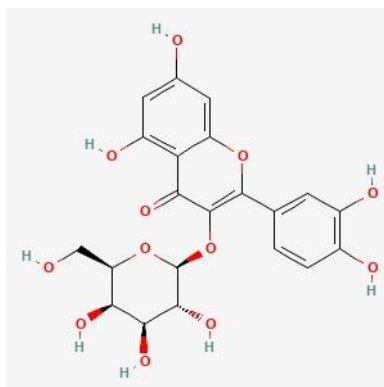


Value of input compound

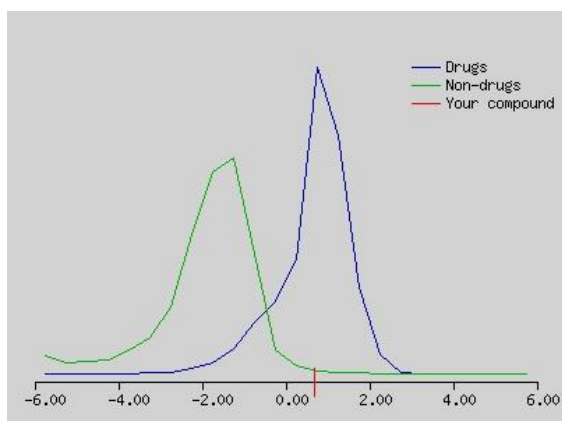
Mean value of dataset

Predicted LD50: 274mg/kg of Azadirachtani

4. Molecular structure of Hyperoside:



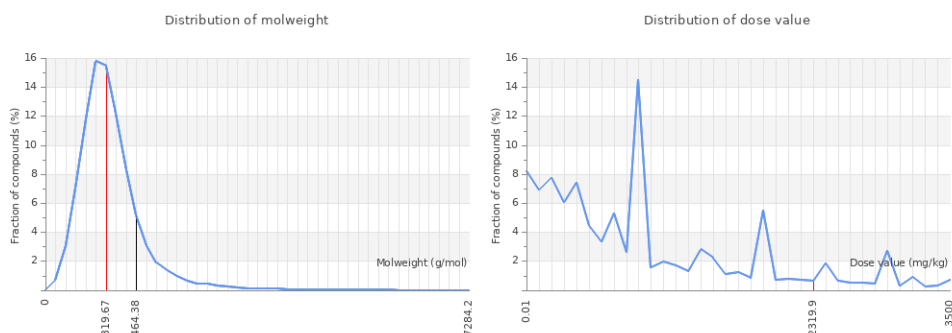
• Drug-likeness model score of Hyperoside : 0.68



➤ Toxicity prediction of Hyperoside:

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.82
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.88
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.76

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.61
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Inactive	0.67
Toxicity end points	<u>Carcinogenicity</u>	carcino	Inactive	0.85
Toxicity end points	<u>Immunotoxicity</u>	immuno	Active	0.66
Toxicity end points	<u>Mutagenicity</u>	mutagen	Inactive	0.76
Toxicity end points	<u>Cytotoxicity</u>	cyto	Inactive	0.69
Toxicity end points	<u>BBB-barrier</u>	bbb	Inactive	0.57
Toxicity end points	<u>Ecotoxicity</u>	eco	Inactive	0.58
Toxicity end points	<u>Clinical toxicity</u>	clinical	Active	0.51
Toxicity end points	<u>Nutritional toxicity</u>	nutri	Active	0.55
Tox21-Nuclear receptor signalling pathways	<u>Aryl hydrocarbon Receptor (AhR)</u>	nr_ahr	Inactive	0.92

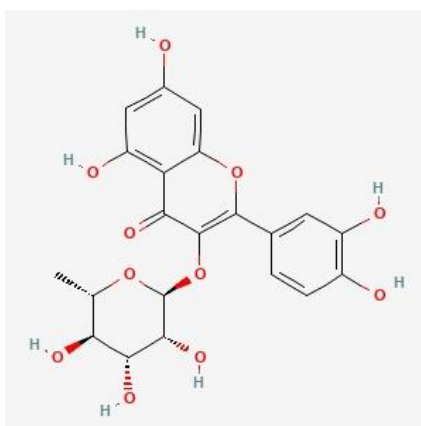


█ Value of input compound

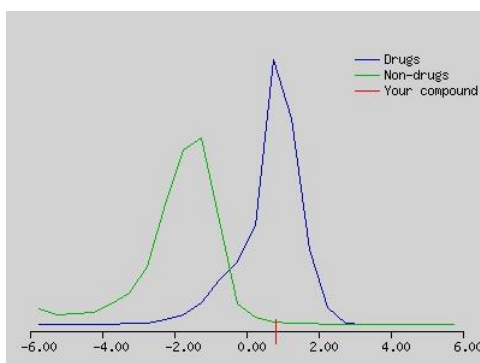
█ Mean value of dataset

Predicted LD50: 5000mg/kg of Hyperoside

➤ Molecular structure of Quercetin:

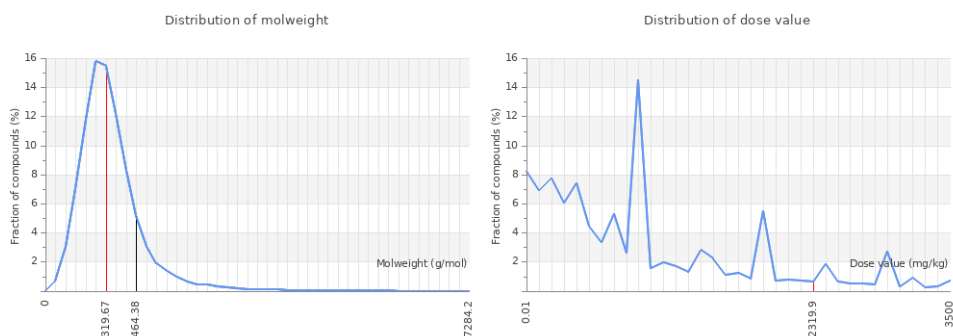


- Drug-likeness model score of Quercetin: 0.82



➤ Toxicity prediction of Quercitrinin:

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.73
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.90
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.68
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.82
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Active	0.51
Toxicity end points	<u>Carcinogenicity</u>	carcino	Active	0.50
Toxicity end points	<u>Immunotoxicity</u>	immuno	Active	0.97
Toxicity end points	<u>Mutagenicity</u>	mutagen	Inactive	0.71
Toxicity end points	<u>Cytotoxicity</u>	cyto	Inactive	0.93
Toxicity end points	<u>BBB-barrier</u>	bbb	Inactive	0.55

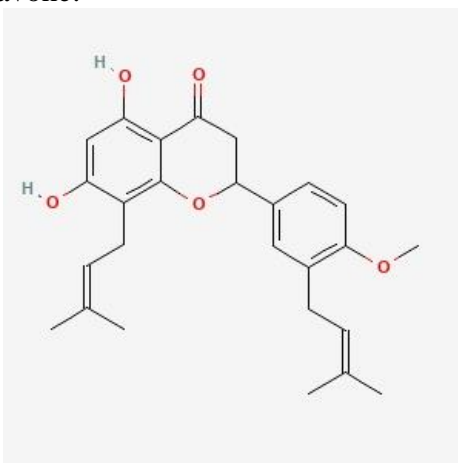


█ Value of input compound

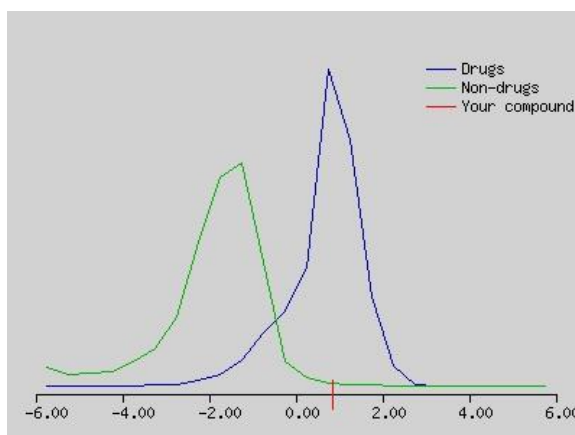
█ Mean value of dataset

Predicted LD50: 5000mg/kg of Quercetrinin

5. Molecular structure of Nimboflavone:

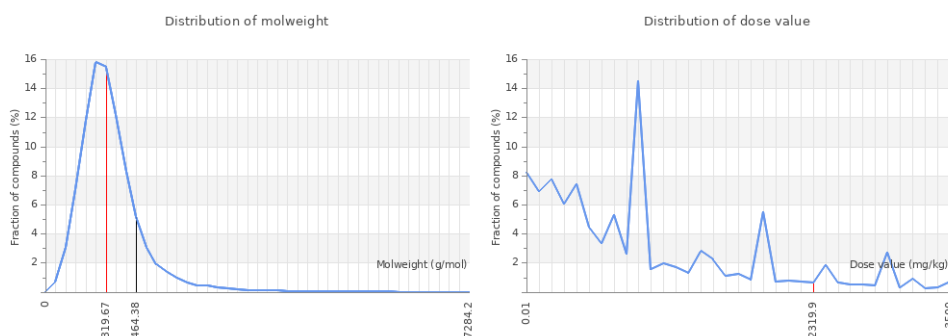


- Drug-likeness model score of Nimboflavone : 0.84



➤ Toxicity prediction of Nimboflavone :

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	<u>Hepatotoxicity</u>	dili	Inactive	0.69
Organ toxicity	<u>Neurotoxicity</u>	neuro	Inactive	0.74
Organ toxicity	<u>Nephrotoxicity</u>	nephro	Active	0.64
Organ toxicity	<u>Respiratory toxicity</u>	respi	Active	0.75
Organ toxicity	<u>Cardiotoxicity</u>	cardio	Inactive	0.88
Toxicity points end	<u>Carcinogenicity</u>	carcino	Inactive	0.67
Toxicity points end	<u>Immunotoxicity</u>	immuno	Active	0.99
Toxicity points end	<u>Mutagenicity</u>	mutagen	Inactive	0.68



Value of input compound

Mean value of dataset

Predicted LD50: 2000mg/kg of Nimboflavone

### 6.3 Network Pharmacology Interpretation

Neem phytoconstituents interact with several molecular targets implicated in inflammation, oxidative stress, immunological modulation, and cancer-related pathways, according to network pharmacology study. Better drug-likeness characteristics were shown by compounds like rutin and quercetin, which may have greater therapeutic potential. The study backs up the idea that, as opposed to single-target interactions, herbal medicines work through multi-component and multi-target mechanisms.

Sr.no	Aspect	Findings	Significance
1.	Phytochemical Richness	7 major secondary metabolites confirmed	Supports neem's broad medicinal use
2.	Lead Compound	Quercetin	Highest activity count (176), good drug-likeness
3.	Safest Compound	Rutin / Hyperoside / Quercitrin	LD50 = 5000 mg/kg; non-toxic
4.	Drug-Likeness	Nimbaflavone (0.84), Rutin (0.91)	High scores; good candidates for drug development
5.	Traditional Validation Use	Multi-target network pharmacology analysis	Scientifically validates Ayurvedic uses of neem
6.	Limitation	Rutin, Azadirachtanin exceed Lipinski's Rule of 5	Bioavailability challenges; formulation needed
7.	Future Direction	Molecular docking, in vivo studies, clinical trials	Next steps for drug development

#### ➤ Future Scope

- Compound–target interactions can be verified using molecular docking experiments.
- Additional evidence of therapeutic efficacy may come from in vivo pharmacological investigations.
- To assess long-term safety, toxicological research is necessary.
- New medicinal agents and herbal formulations may be created using neem phytoconstituents.
- Human therapeutic applications can be assessed through clinical research.

### 7. CONCLUSION

Using network pharmacology analysis and phytochemical screening, the current study effectively illustrated the medicinal potential of *Azadirachta indica* leaf phytoconstituents. Important bioactive

substances that support antioxidant, antibacterial, anti-inflammatory, and immunomodulatory properties, such as alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids, were found, according to preliminary research. Quercetin, rutin, hyperoside, and nimbaflavone were found to be promising compounds for therapeutic development with favorable safety profiles by drug-likeness and pharmacokinetic evaluation. Neem phytoconstituents act through a variety of biological targets and pathways linked to inflammation, oxidative stress, immunology, and the development of cancer, according to network pharmacology study. Overall, the results demonstrate neem's potential in the development of natural drugs and provide scientific evidence for its traditional medical benefits. For therapeutic validation, more molecular docking, in vivo research, and clinical trials are advised.

## REFERENCES:

1. Sarkar S, Singh RP, Bhattacharya G. Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. *3 Biotech*. 2021 Apr;11(4):178.
2. Nagini S, Palrasu M, Bishayee A. Limonoids from neem (*Azadirachta indica* A. Juss.) are potential anticancer drug candidates. *Medicinal research reviews*. 2024 Mar;44(2):457-96.
3. Alzohairy MA. Therapeutics role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*. 2016;2016(1):7382506.
4. Gupta SC, Prasad S, Tyagi AK, Kunnumakkara AB, Aggarwal BB. Neem (*Azadirachta indica*): An indian traditional panacea with modern molecular basis. *Phytomedicine*. 2017 Oct 15;34:14-20.
5. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of *Azadirachta indica*. *Current Science*. 2002;82(11):1336–1345.
6. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. *Current Medicinal Chemistry – Anti-Cancer Agents*. 2005;5(2):149–156.
7. Mohammad A. Alzohairy. Therapeutic role of *Azadirachta indica* (Neem) and their active constituents in disease prevention and treatment. *Evidence-Based Complementary and Alternative Medicine*. 2016;2016:7382506.
8. Paul R, Prasad M, Sah NK. Anticancer biology of neem leaf constituents: A review. *Cancer Biology & Therapy*. 2011;12(6):467–476.
9. Andrew L. Hopkins. Network pharmacology: The next paradigm in drug discovery. *Nature Chemical Biology*. 2008;4(11):682–690.
10. Li S, Zhang B. Traditional medicine network pharmacology: Theory and applications. *Chinese Journal of Natural Medicines*. 2013;11(2):110–120.
11. Sharma P, Tomar L, Bachwani M, Bansal V. Review on neem leaves and medicinal applications. *International Research Journal of Pharmacy*. 2011;2(12):97–102.
12. Roy A, Saraf S. Limonoids and pharmacological importance in medicinal plants. *Biological and Pharmaceutical Bulletin*. 2006;29(2):191–201.
13. Kharwar RN, Upadhyay R, Dubey NK, Raghuwanshi R. Medicinal applications of neem leaves. *Journal of Applied Pharmaceutical Science*. 2020;10(4):1–10.
14. Zhang R, Zhu X, Bai H, Ning K. Network pharmacology databases for herbal medicine research. *Briefings in Bioinformatics*. 2019;20(4):1524–1536.
15. Trease and Evans' Pharmacognosy, Evans WC. *Trease and Evans Pharmacognosy*. 16th ed. Saunders Elsevier; 2009.
16. Practical Pharmacognosy, Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments*. 19th ed. Nirali Prakashan; 2008.
17. Pharmacognosy, Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 54th ed. Nirali Prakashan; 2014.

18. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. Chapman and Hall; 1998.
19. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Spectrum Books Ltd; 1993.
20. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. 2005;4(7):685–688.
21. IMPPAT Database
22. TCMSP Database
23. Additional information regarding phytochemical screening and pharmacological properties of *Azadirachta indica* was collected from published review articles and standard medicinal plant literature.
24. Indian Medicinal Plants, Kirtikar KR, Basu BD. *Indian Medicinal Plants*. 2nd ed. International Book Distributors; 2005.
25. Ghimeray AK, Jin C, Ghimire BK, Cho DH. Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica*. *African Journal of Biotechnology*. 2009;8(13):3084–3091.
26. Govindachari TR. Chemical and biological investigations on *Azadirachta indica* (the neem tree). *Current Science*. 1992;63(3):117–122.
27. Bhowmik D, Chiranjib YJ, Tripathi KK, Kumar KS. Herbal remedies of *Azadirachta indica* and its medicinal application. *Journal of Chemical and Pharmaceutical Research*. 2010;2(1):62–72.
28. Chattopadhyay RR. Possible mechanism of antihyperglycemic effect of neem leaf extract. *Journal of Ethnopharmacology*. 1996;67(3):373–376.
29. Boeke SJ, Boersma MG, Alink GM, et al. Safety evaluation of neem (*Azadirachta indica*) derived pesticides. *Journal of Ethnopharmacology*. 2004;94(1):25–41.
30. Pandey G, Verma KK, Singh M. Evaluation of phytochemical, antibacterial and free radical scavenging properties of neem leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014;6(2):444–447.