International Journal on Science and Technology (IJSAT)

E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

Evaluation of Pharmacological Potential of Punica granatum Leaves Using in Silico Approaches

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Abstract:

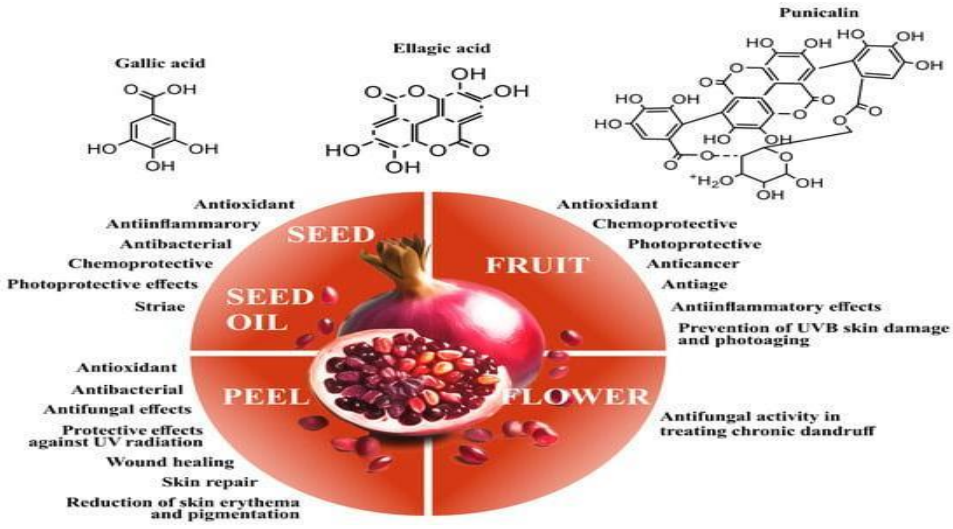
The present study was carried out to investigate the phytochemical constituents and pharmacological potential of Punica granatum leaves using a network pharmacology approach. *Punica granatum*, commonly known as pomegranate or Dalimb, is a medicinal plant widely used in traditional systems of medicine due to its therapeutic properties. Fresh leaves were collected, shade dried, powdered, and extracted using suitable solvents for phytochemical analysis. Preliminary phytochemical screening revealed the presence of important bioactive compounds such as flavonoids, tannins, alkaloids, glycosides, saponins, phenolic compounds, and terpenoids. These phytoconstituents are associated with various pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, and anticancer effects. Network pharmacology analysis was performed using bioinformatics databases and software tools such as SwissTargetPrediction, GeneCards, STITCH, and Cytoscape to identify compound-target interactions and biological pathways. The study suggested that the therapeutic effects of *Punica granatum* leaves are mediated through multi-component and multi-target mechanisms. The findings support the traditional medicinal uses of the plant and indicate its potential for future drug discovery and development of herbal therapeutic agents

Punica granatum Leaves → Phytochemical Screening → Target Prediction → Network Analysis → Pathway Enrichment → Therapeutic Mechanism

Keywords: *Punica granatum*, Network Pharmacology, Phytoconstituents, Bioactive Compounds, ADME Analysis, Medicinal Plants, Systems Pharmacology

Introduction:

Introduction of Plant:



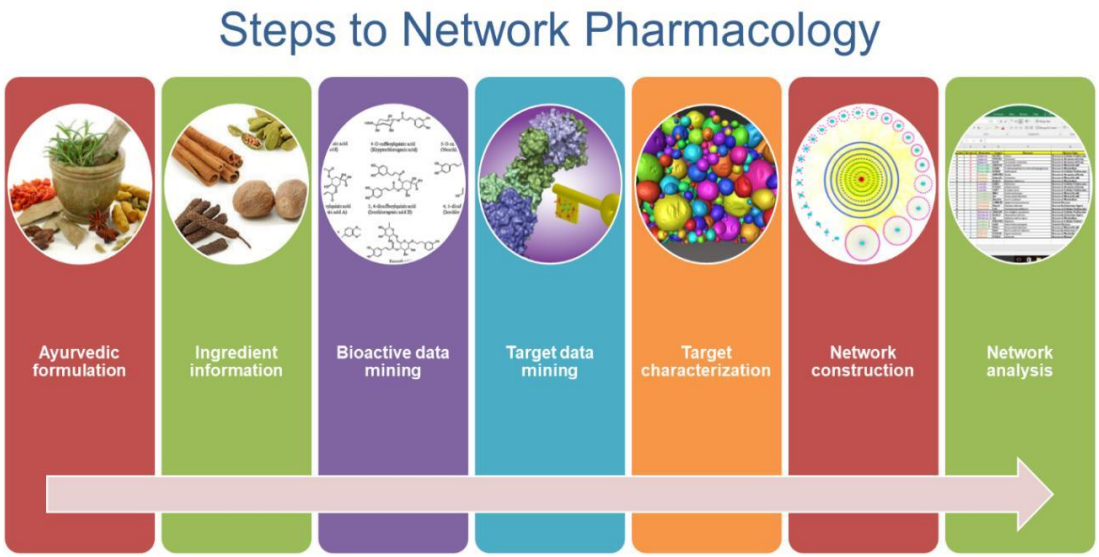
Punica granatum, commonly known as pomegranate and locally called *Dalimb*, is an important medicinal plant widely used in traditional as well as modern systems of medicine. The plant belongs to the family **Lythraceae** and has been cultivated for several centuries because of its nutritional, therapeutic, and economic importance. It is believed to have originated in Iran and neighboring regions and is now extensively distributed in tropical and subtropical countries including India, China, Afghanistan, Turkey, and Mediterranean regions.In India, pomegranate is cultivated on a large scale, especially in Maharashtra, Karnataka, Gujarat, Rajasthan, and Andhra Pradesh. Maharashtra is considered one of the leading producers of pomegranate due to suitable climatic conditions. The plant is highly drought tolerant and can survive in semi-arid environments. Because of its medicinal significance and rich phytochemical profile, *Punica granatum* has attracted increasing scientific attention in the field of pharmacology and phytomedicine.The pomegranate plant is a deciduous shrub or small tree that grows approximately 5–10 meters in height. The plant possesses thorny branches with glossy narrow leaves and attractive reddish flowers. The fruit is round in shape with a thick leathery rind enclosing numerous edible seeds surrounded by juicy arils. Almost every part of the plant including leaves, peel, bark, flowers, roots, and seeds has been reported to possess medicinal properties. Among these, the leaves are considered a rich source of bioactive phytoconstituents with promising therapeutic applications.

Traditionally, pomegranate leaves have been used in Ayurveda and folk medicine for the treatment of various disorders such as inflammation, diabetes, microbial infections, wounds, diarrhea, and oxidative stress-related diseases. Decoctions and extracts prepared from the leaves have been utilized for their antimicrobial, antioxidant, and anti-inflammatory properties.

Phytochemical Constituent	**Part of Plant**	**Activity**	**Pharmacological Action**
Punicalagin	Peel, Leaves	Antioxidant	Free radical scavenging activity
Ellagic Acid	Peel, Leaves	Anticancer	Inhibits tumor cell growth
Gallic Acid	Leaves, Fruit	Anti-inflammatory	Reduces inflammatory mediators
Quercetin	Leaves	Antioxidant	Protects cells from oxidative stress
Kaempferol	Leaves, Flowers	Antimicrobial	Inhibits microbial growth
Tannins	Peel, Bark	Antidiarrheal	Astringent action on intestinal mucosa
Flavonoids	Leaves,	Cardioprotective	Improves blood circulation

	Fruits		
Alkaloids	Root Bark	Anthelmintic	Paralysis of intestinal worms
Saponins	Leaves	Immunomodulatory	Enhances immune response
Polyphenols	Fruit, Leaves	Hepatoprotective	Protects liver cells from damage

Network Pharmacology Introduction:



Network pharmacology is an emerging interdisciplinary field that integrates pharmacology, bioinformatics, systems biology, computational biology, and molecular biology to understand the interactions between drugs, targets, genes, proteins, and diseases. The concept of network pharmacology was first introduced to overcome the limitations of the traditional "one drug-one target-one disease" approach. Unlike conventional synthetic drugs that generally act on a single molecular target, herbal medicines contain multiple bioactive compounds capable of interacting with several biological targets simultaneously. Therefore, network pharmacology provides a holistic approach for studying the complex mechanisms of herbal medicines and their therapeutic effects.

The basic principle of network pharmacology is based on the interaction among compounds, target proteins, signaling pathways, and diseases through biological networks. It helps in identifying active phytoconstituents, predicting target proteins, understanding pathway interactions, and exploring molecular mechanisms involved in disease treatment. This approach combines experimental pharmacology with computational analysis and database screening to provide scientific validation for traditional medicinal systems.

Network pharmacology has gained significant importance in herbal drug research because medicinal plants contain diverse phytochemicals that exhibit synergistic therapeutic effects. By constructing compound-target-pathway networks, researchers can identify how multiple phytoconstituents regulate various biological pathways associated with diseases such as cancer, diabetes, inflammation, cardiovascular disorders, and neurodegenerative diseases. The approach also assists in discovering novel drug candidates and understanding adverse drug reactions.

Introduction of Softwares:

1. Dr. Duke's Phytochemical and Ethnobotanical Databases

Dr. Duke's Phytochemical and Ethnobotanical Databases is an online database developed by Dr. James A. Duke under the United States Department of Agriculture (USDA). The database provides comprehensive information regarding medicinal plants, phytochemical constituents, biological activities, ethnobotanical uses, and therapeutic properties of natural compounds. It is widely used in phytochemistry, pharmacognosy, and network pharmacology research for identification of bioactive compounds present in medicinal plants.

2. Molsoft

Molsoft is a computational chemistry and molecular modeling software platform used in drug discovery and bioinformatics research. It provides tools for molecular docking, virtual screening, protein structure prediction, and calculation of molecular properties. Molsoft software is useful for evaluating drug-likeness, molecular descriptors, and pharmacokinetic properties of phytoconstituents during network pharmacology studies.

3. SwissADME

SwissADME is a free online web tool used for prediction of pharmacokinetic properties, drug-likeness, physicochemical parameters, and medicinal chemistry friendliness of compounds. It helps researchers evaluate absorption, distribution, metabolism, and excretion (ADME) properties of phytochemicals. SwissADME also predicts Lipinski's Rule of Five, gastrointestinal absorption, blood-brain barrier permeability, and bioavailability of compounds.

4. ProTox-II

ProTox-II is an online toxicity prediction platform used for predicting toxicity profiles of chemical compounds. It estimates hepatotoxicity, carcinogenicity, cytotoxicity, immunotoxicity, mutagenicity, and LD50 values using machine learning and molecular similarity methods. In network pharmacology studies, ProTox-II is used to evaluate the safety and toxicity of phytoconstituents before further drug development studies.

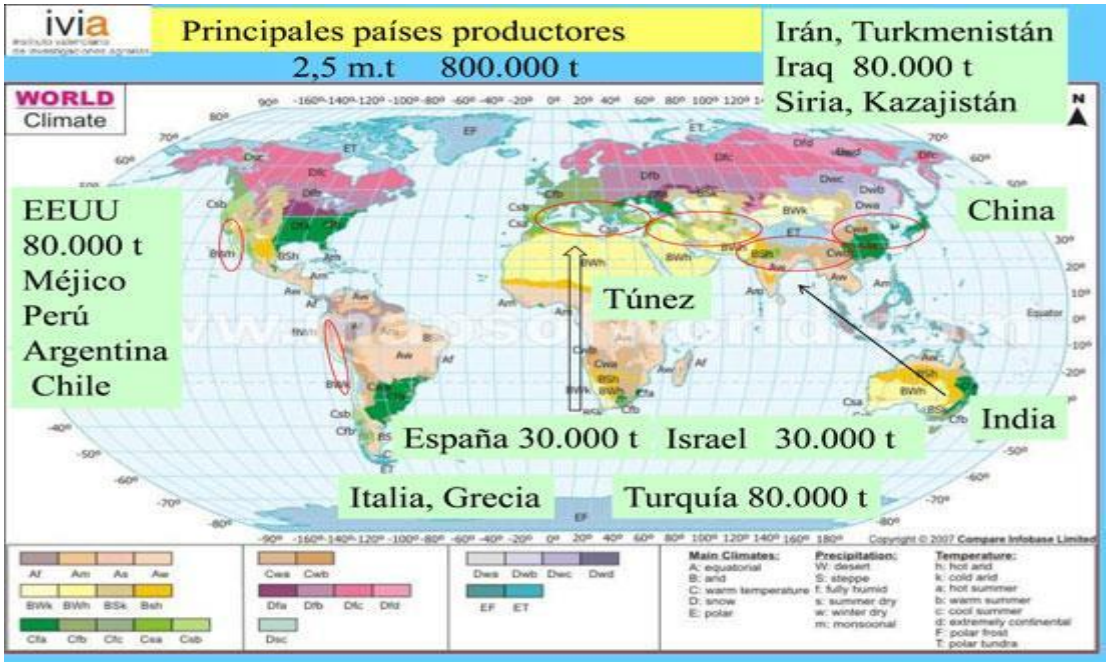
5. IMPPAT Database

IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) is a comprehensive database containing information about Indian medicinal plants, phytochemicals, and their therapeutic uses. It provides detailed data regarding plant constituents, chemical structures, biological activities, and traditional medicinal applications. IMPPAT is widely used in network pharmacology research for identification and screening of phytoconstituents from medicinal plants.

Taxonomical Classification of Punica Granatum:

Sr. No.	Taxonomical Rank	Classification
1	Kingdom	Plantae
2	Subkingdom	Tracheobionta
3	Division	Magnoliophyta
4	Class	Magnoliopsida
5	Subclass	Rosidae
6	Order	Myrtales
7	Family	Lythraceae
8	Genus	Punica
9	Species	*Punica granatum* L.

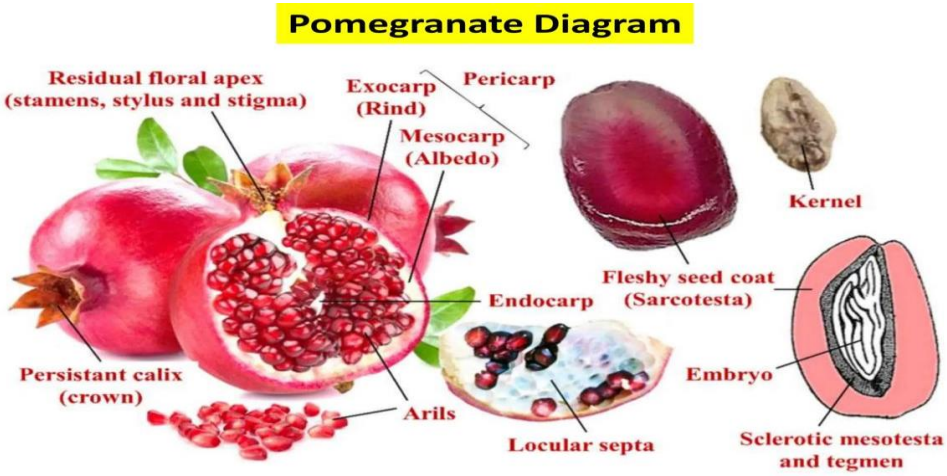
Geographical Distribution:



Punica granatum is believed to have originated in Iran and surrounding regions and is now widely distributed in tropical and subtropical parts of the world. The plant is extensively cultivated in countries such as India, Iran, Afghanistan, China, Turkey, Spain, and Mediterranean regions.

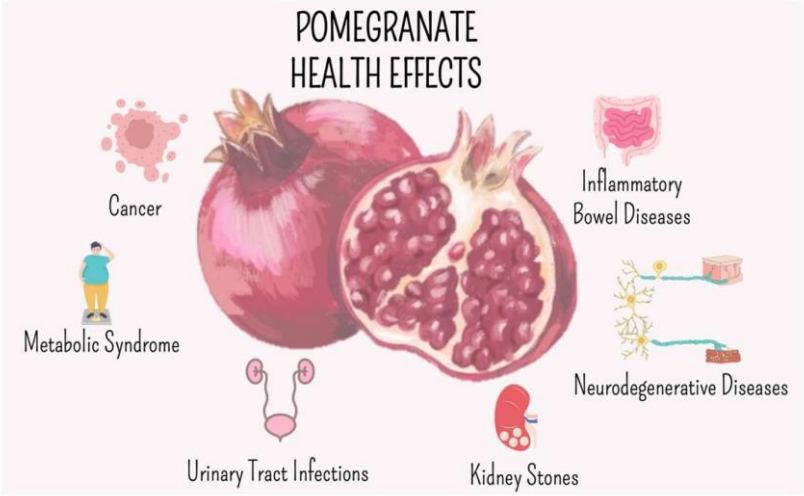
In India, pomegranate is mainly grown in Maharashtra, Karnataka, Gujarat, Rajasthan, and Andhra Pradesh due to favorable climatic conditions. The plant grows well in semi-arid regions and is highly tolerant to drought conditions.

Morphological Features:



Punica granatum is a deciduous shrub or small tree that usually grows up to 5–10 meters in height. The plant possesses numerous spiny and branched stems with smooth reddish-brown bark. Young branches are angular while older branches become woody with age.The leaves are simple, glossy, narrow, and lanceolate to oblong in shape. They are arranged oppositely or sometimes alternately on the branches and measure about 3–7 cm in length. Young leaves appear reddish in color and gradually become dark green upon maturation.The flowers are bright red or orange-red, bisexual, and tubular in shape. They usually occur singly or in clusters at the tips of branches. Flowering commonly takes place during spring and summer seasons.The fruit is a round berry with a hard leathery rind enclosing numerous seeds. Each seed is surrounded by a juicy edible aril which varies in color from pink to deep red. The roots are hard and woody, while the bark contains medicinally important alkaloids.

Review of Liturature:
Medicinal Uses of Punica Granatum:



1. Used as an antioxidant to reduce oxidative stress and free radical damage.
2. Exhibits anti-inflammatory activity and helps in the treatment of inflammation-related disorders.
3. Possesses antimicrobial activity against bacterial and fungal infections.
4. Used in the management of diabetes by helping regulate blood glucose levels.
5. Helps in the treatment of diarrhea, dysentery, and other gastrointestinal disorders.
6. Shows cardioprotective activity by reducing cholesterol and improving heart health.

7. Possesses hepatoprotective activity and protects the liver from toxic damage.
8. Used in wound healing due to its antimicrobial and tissue repair properties.
9. Exhibits anticancer activity against various cancer cell lines because of its polyphenolic compounds.
10. Used in oral and dental care for treating mouth ulcers, gum infections, and dental plaque.
11. Helps boost immunity due to the presence of bioactive phytochemicals and antioxidants.
12. Traditionally used for the treatment of cough, fever, and throat infections

Pharmacological Activities:

 Antioxidant Activity:
Punica granatum possesses strong antioxidant activity due to the presence of flavonoids, tannins, and polyphenols which help in scavenging free radicals and reducing oxidative stress.
 Anti-inflammatory Activity:
The plant inhibits inflammatory mediators and enzymes, thereby helping in the management of inflammatory disorders.
 Antimicrobial Activity:
Extracts of pomegranate leaves, peel, and bark exhibit antibacterial and antifungal activities against various pathogenic microorganisms.
 Antidiabetic Activity:
Pomegranate helps in lowering blood glucose levels and improving insulin sensitivity due to its bioactive phytoconstituents.
 Anticancer Activity:
Polyphenolic compounds present in the plant show inhibitory effects against different cancer cell lines by inducing apoptosis and suppressing tumor growth.
 Cardioprotective Activity:
The antioxidant compounds help in reducing cholesterol oxidation, improving blood circulation, and protecting against cardiovascular diseases.
 Hepatoprotective Activity:
Pomegranate extracts protect liver cells from toxin-induced damage and improve liver function.
 Wound Healing Activity:
The plant promotes tissue repair and accelerates wound healing because of its antimicrobial and anti-inflammatory properties.
 Gastroprotective Activity:
Pomegranate is used in the treatment of ulcers, diarrhea, dysentery, and other gastrointestinal disorders.
 Immunomodulatory Activity:
Bioactive constituents present in the plant help enhance immune response and improve body defense mechanisms

Other Uses:
 Food Industry:
Pomegranate fruits are widely used in the preparation of juices, syrups, jams, jellies, beverages, and flavoring agents.
 Nutritional Supplement:
The fruit is consumed as a health supplement because it is rich in vitamins, minerals, and antioxidants.
 Cosmetic Industry:
Pomegranate extracts are used in creams, lotions, shampoos, and anti-aging cosmetic products due to their antioxidant properties.
 Natural Coloring Agent:
The peel and flowers are used as natural dyes for fabrics and food products.

 Agricultural Use:
Pomegranate peel and plant residues are utilized as organic manure and animal feed.
 Traditional Religious Use:
The fruit is considered sacred in several cultures and is used during religious ceremonies and traditional rituals.
 Commercial Importance:
Pomegranate cultivation contributes significantly to the agricultural economy because of high market demand and export value.

Aim:
The main aim of the present study is to investigate the phytochemical constituents and pharmacological potential of Punica granatum leaves using a network pharmacology approach in order to identify bioactive compounds, target proteins, and biological pathways involved in therapeutic activities.
Objectives:
1. **To collect and authenticate the plant material**
To collect fresh leaves of *Punica granatum* and authenticate them for correct botanical identification before experimental studies.
2. **To prepare and process plant extract**
To prepare powdered leaf material and obtain extract using suitable extraction methods for phytochemical and pharmacological analysis.
3. **To perform preliminary phytochemical screening**
To identify the presence of important phytochemical constituents such as flavonoids, tannins, alkaloids, glycosides, saponins, phenolic compounds, and terpenoids in the leaf extract.

Plan of Work:

Literature Survey
↓
Collection of Plant Material
↓
Authentication of Plant Material
↓
Drying and Powder Preparation
↓
Preparation of Extract
↓
Preliminary Phytochemical Screening
↓
Identification of Bioactive Compounds
↓
ADME and Toxicity Prediction
(SwissADME and ProTox-II Analysis)
↓

Target Protein Identification
↓
Network Pharmacology Analysis
↓
Compound–Target Network Construction
↓
Pathway Enrichment Analysis
↓
Molecular Interaction Studies
↓
Interpretation and Data Analysis

Methodology:

Collection and Preparation of Plant Material:

Fresh leaves of Punica granatum were collected from local agricultural fields/gardens in Maharashtra, India. The collected leaves were washed thoroughly with distilled water to remove dust and impurities. The plant material was authenticated by a botanist for proper identification. The leaves were shade dried at room temperature for 7–10 days and then powdered using a mechanical grinder. The powdered material was stored in an airtight container for further experimental studies.

Preparation of Punica Granatum Leaf Extract:

Fresh leaves of Punica granatum were collected from local agricultural fields/gardens in Maharashtra, India. The collected leaves were washed thoroughly with distilled water to remove dust and impurities. The plant material was authenticated by a botanist for proper identification. The leaves were shade dried at room temperature for 7–10 days and then powdered using a mechanical grinder. The powdered material was stored in an airtight container for further experimental studies.

Maceration Method:

In the maceration method, dried neem leaf powder of Azadirachta indica was soaked in ethanol or methanol in a closed container for about 72 hours at room temperature. The mixture was shaken occasionally to enhance the extraction of phytochemical constituents from the plant material into the solvent.

After the extraction period, the mixture was filtered using muslin cloth or Whatman filter paper to remove the solid residue. The collected filtrate was then concentrated by evaporating the solvent under reduced pressure or at low temperature to obtain a crude neem leaf extract rich in bioactive compounds. The extract was finally stored in airtight containers for further phytochemical analysis**.**

Preliminary Phytochemical Screening:

Sr. No.	Phytochemical Constituents	Test Performed	Observation	Result
1	Alkaloids	Mayer's Test	Cream colored precipitate	Present (+)

2	Flavonoids	Alkaline Reagent Test	Yellow coloration	Present (+)
3	Tannins	Ferric Chloride Test	Blue-black coloration	Present (+)
4	Saponins	Foam Test	Stable foam formation	Present (+)
5	Glycosides	Keller-Killiani Test	Brown ring formation	Present (+)
6	Phenolic Compounds	Ferric Chloride Test	Dark blue coloration	Present (+)
7	Terpenoids	Salkowski Test	Reddish-brown interface	Present (+)
8	Proteins	Biuret Test	Violet coloration	Absent (–)
9	Carbohydrates	Molisch's Test	Violet ring formation	Present ((+)

Phytochemical Screening Results:

Preliminary phytochemical screening of the leaf extract of Punica granatum revealed the presence of several important bioactive constituents such as flavonoids, tannins, alkaloids, saponins, glycosides, phenolic compounds, and terpenoids. These phytochemicals are known for their antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities. The presence of phenolic compounds and flavonoids indicates strong antioxidant potential of the plant. The obtained results support the traditional medicinal use of *Punica granatum* leaves and suggest their potential importance in network pharmacology studies for multi-target therapeutic applications.

Phytoconstituent Analysis:

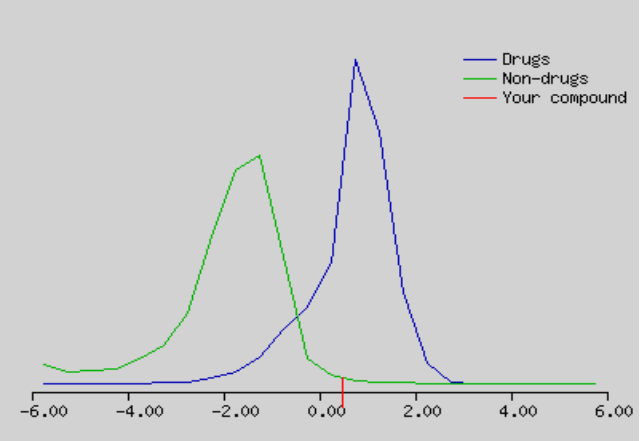
Chemical name	Activity	Body part	Molecular weight	HBD	HBA	druglikeliness score	
Kaempferol	5	Leaves	286.24g/mol	4	6	0.78	
Luteolin	5	Leaves	286.24	4	6	0.74	
Gallic Acid	4	Peel,Leaves	170.12	4	5	0.86	
Quercetin	6	Leaves	302.24	5	7	0.75	

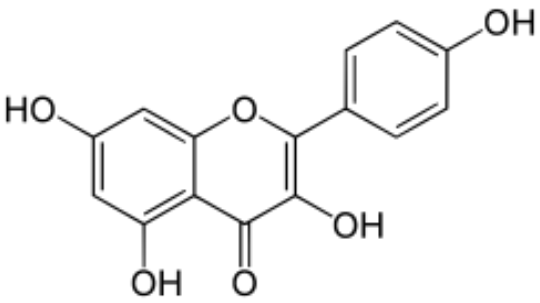
DaucosterOl	5	Leaves	576.85	4	6	0.52	

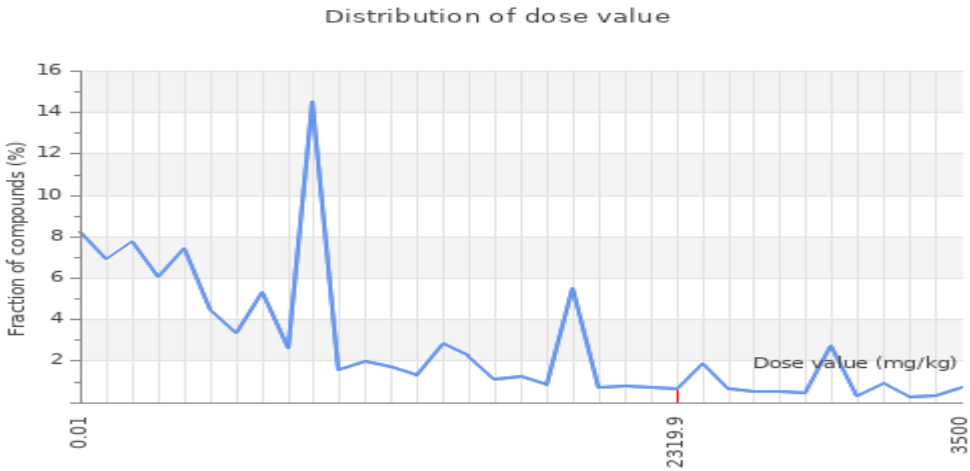
KAEMFEROL:

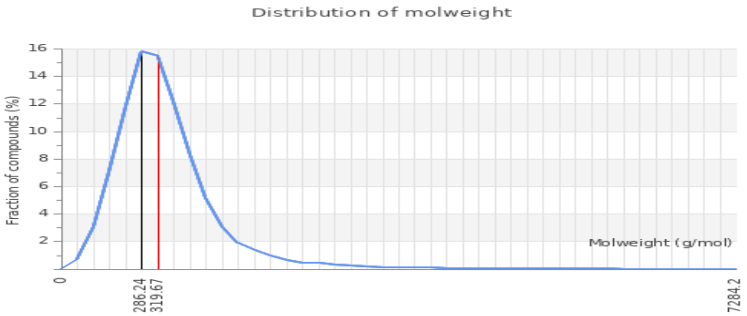
Predicted LD50: 3919mg/kg
Predicted Toxicity Class: 5
Average similarity: 82.46%
Prediction accuracy: 70.97%

Drug-likenessmodelscore: 0.50









Toxicity Model Report

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Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.68
Organ toxicity	Nephrotoxicity	nephro	Active	0.62
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.91
Toxicity end points	Carcinogenicity	carcino	Inactive	0.72
Toxicity end points	Immunotoxicity	immuno	Inactive	0.96
Toxicity end points	Mutagenicity	mutagen	Inactive	0.52
Toxicity end points	Cytotoxicity	cyto	Inactive	0.98
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	**Active**	0.96
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	**Active**	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	**Active**	1.0
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.92
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.92
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.68
Metabolism	Cytochrome CYP1A2	CYP1A2	**Active**	0.93
Metabolism	Cytochrome CYP2C19	CYP2C19	Active	0.65

Classification	Target	Shorthand	Prediction	Probability
Metabolism	Cytochrome CYP2D6	CYP2D6	Active	0.62
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.65

LUTEOLIN:

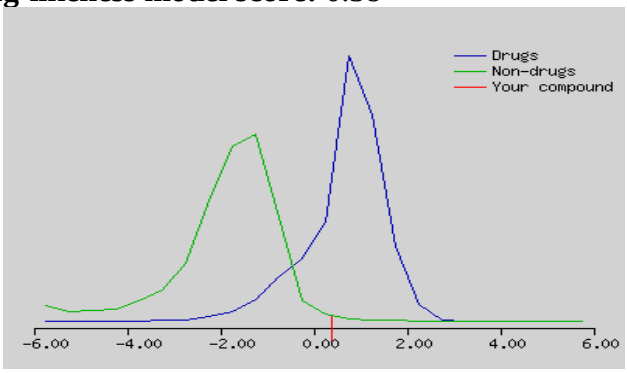
Predicted LD50: 3919mg/kg

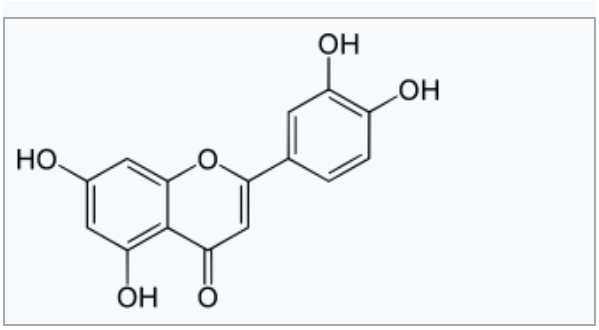
Predicted Toxicity Class: 5

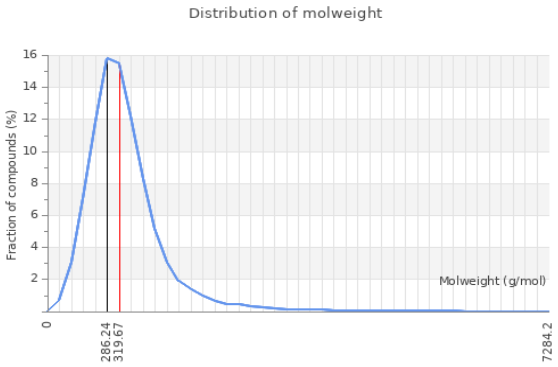
Average similarity: 80.53%

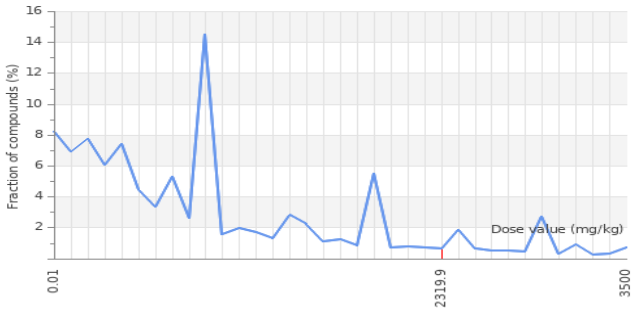
Prediction accuracy: 70.97%

Drug-likeness model score: 0.38









Toxicity Model Report

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Toxicity end points	Carcinogenicity	carcino	Active	0.68
Toxicity end points	Immunotoxicity	immuno	Inactive	0.97
Toxicity end points	Mutagenicity	mutagen	Active	0.51
Toxicity end points	Cytotoxicity	cyto	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.91
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	**Active**	0.87

Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.98
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	**Active**	1.0
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.69
Metabolism	Cytochrome CYP1A2	CYP1A2	**Active**	1.0
Metabolism	Cytochrome CYP2C9	CYP2C9	**Active**	0.99

Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.79

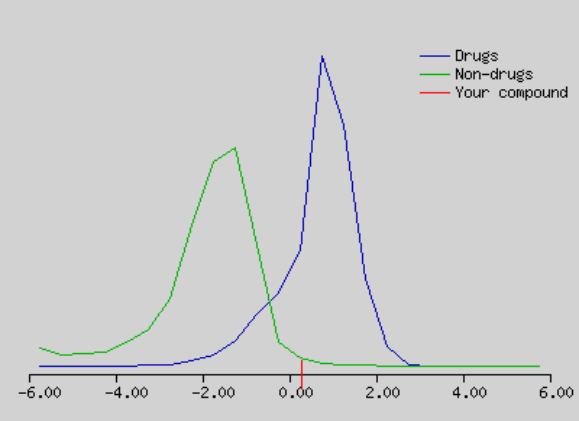
GALLIC ACID:

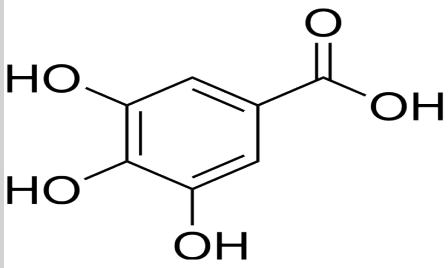
Predicted LD50: 2000mg/kg

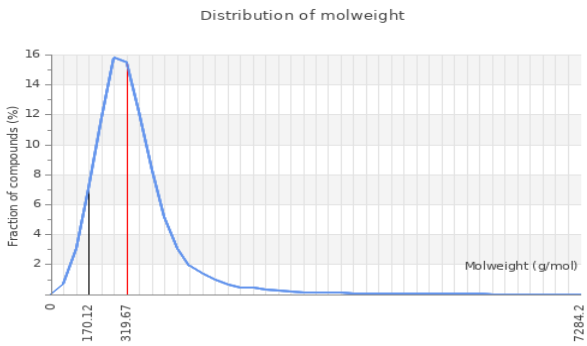
Predicted Toxicity Class: 4

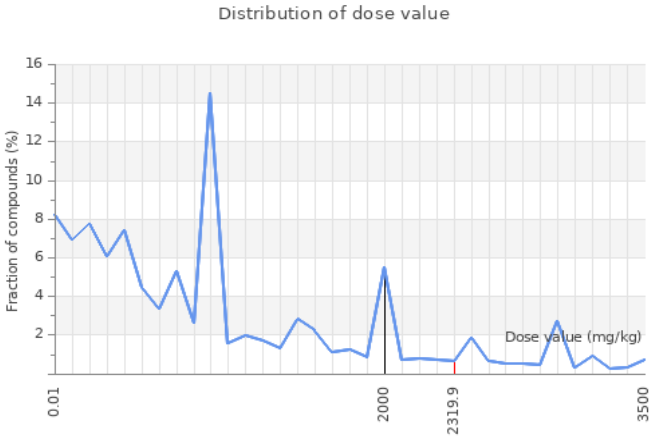
Average similarity: 84.82%
Prediction accuracy: 70.97%

Drug-likeness model score: 0.29









Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.61
Organ toxicity	Nephrotoxicity	nephro	Active	0.69
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.89
Toxicity end points	Carcinogenicity	carcino	Active	0.56
Toxicity end points	Immunotoxicity	immuno	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.94
Toxicity end points	Cytotoxicity	cyto	Inactive	0.91
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.89

Classification	Target	Shorthand	Prediction	Probability
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.99
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.85
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.97
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.97
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.61
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.97
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.88
Metabolism	Cytochrome CYP2C9	CYP2C9	Active	0.51
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.99

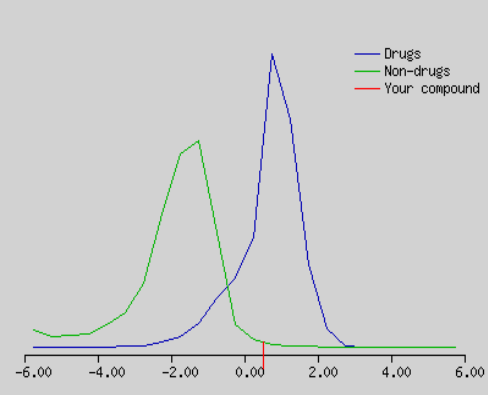
QUERCETIN:

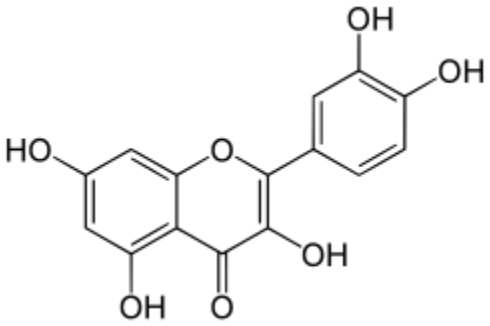
Predicted LD50: 159mg/kg

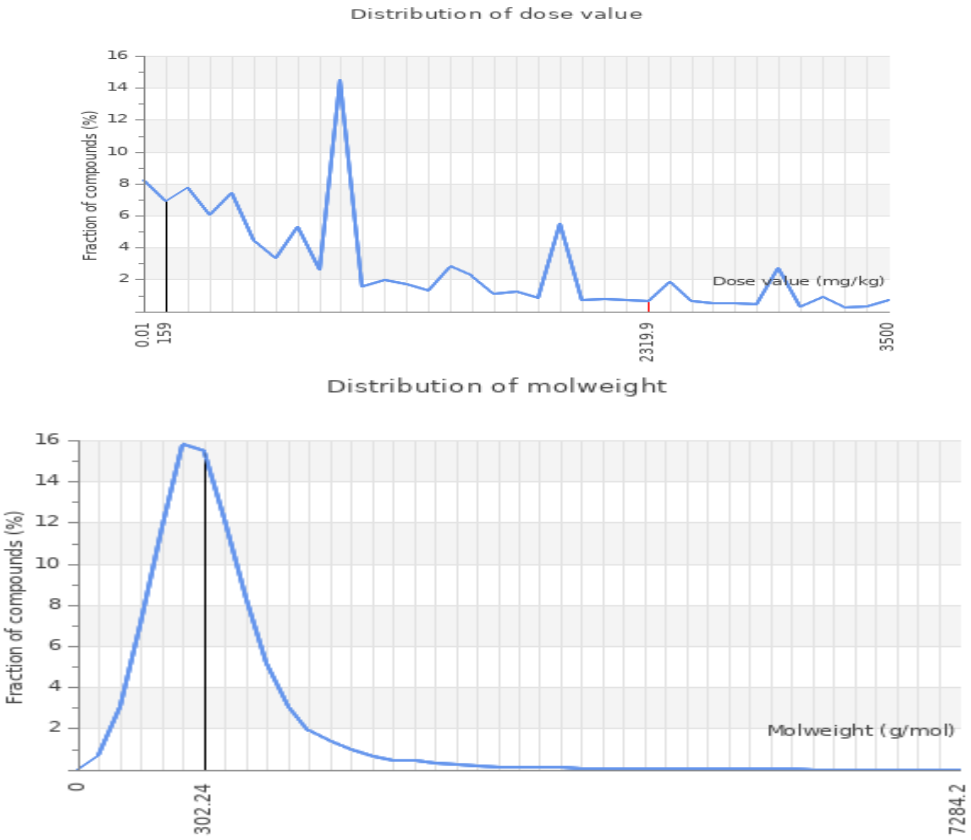
Predicted Toxicity Class: 3

Average similarity: 100%
Prediction accuracy: 100%

Drug-likeness model score: 0.52







Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.69
Organ toxicity	Neurotoxicity	neuro	Inactive	0.89
Organ toxicity	Nephrotoxicity	nephro	Active	0.62
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.99
Toxicity end points	Carcinogenicity	carcino	Active	0.68
Toxicity end points	Immunotoxicity	immuno	Inactive	0.87
Toxicity end points	Mutagenicity	mutagen	Active	0.51
Toxicity end points	Cytotoxicity	cyto	Inactive	0.99
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.99

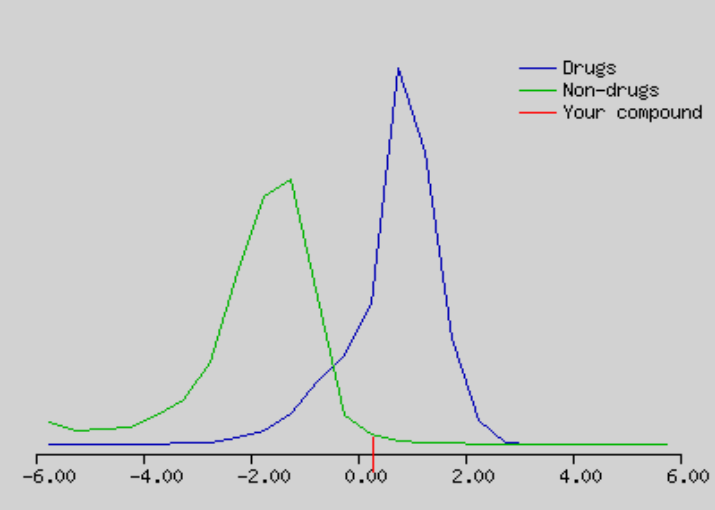
Classification	Target	Shorthand	Prediction	Probability
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.91
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	**Active**	0.87
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.98
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	**Active**	1.0
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.97
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.96
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.92
Molecular Initiating Events	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (AMPAR)	mie_ampar	Inactive	0.97
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.69
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.97
Metabolism	Cytochrome CYP1A2	CYP1A2	**Active**	1.0
Metabolism	Cytochrome CYP2C19	CYP2C19	**Active**	0.77

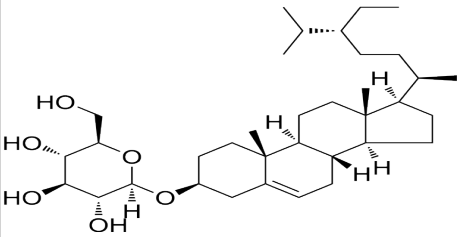
Classification	Target	Shorthand	Prediction	Probability
Metabolism	Cytochrome CYP2C9	CYP2C9	**Active**	0.99
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.85
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.79

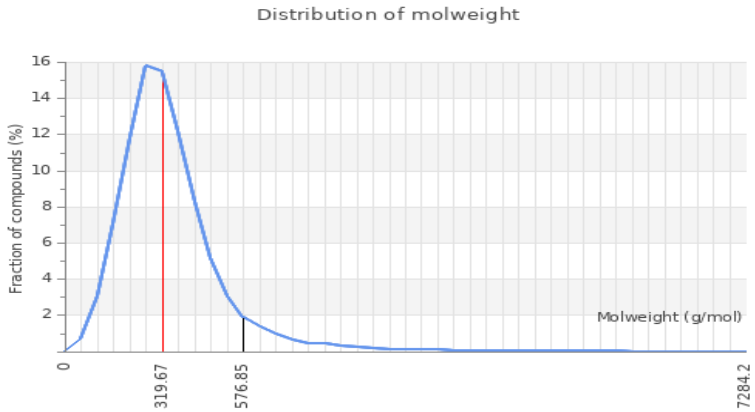
DAUCOSTEROL:
Predicted LD50: 8000mg/kg
Predicted Toxicity Class: 6

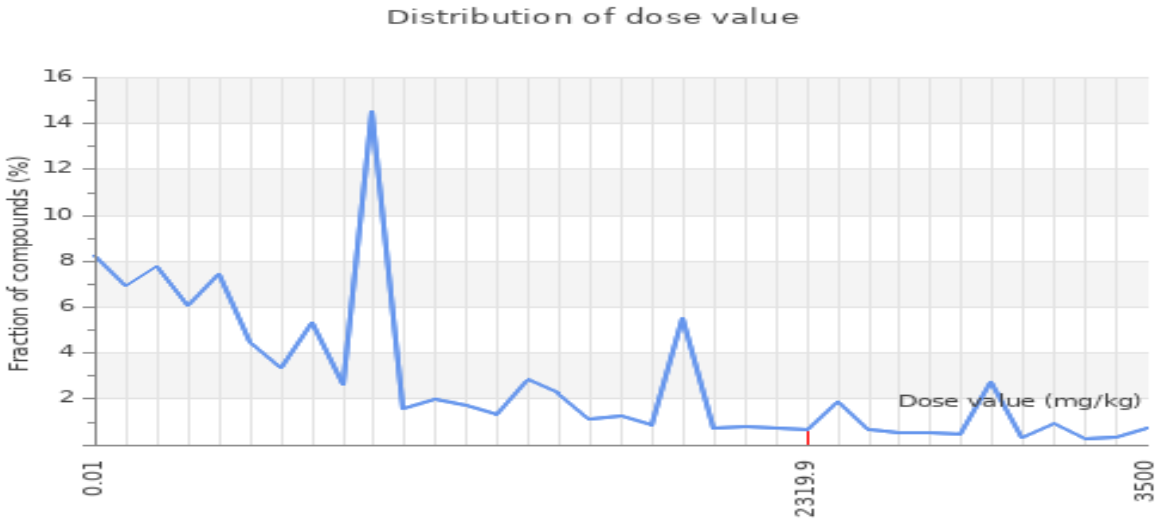
Average similarity: 79.06%
Prediction accuracy: 69.26%

Drug-likeness model score: 0.29









Toxicity Model Report

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.94
Organ toxicity	Neurotoxicity	neuro	Inactive	0.91
Organ toxicity	Cardiotoxicity	cardio	**Active**	0.89
Toxicity end points	Carcinogenicity	carcino	Inactive	0.71
Toxicity end points	Immunotoxicity	immuno	**Active**	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.83
Toxicity end points	Cytotoxicity	cyto	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.80
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.78
Tox21-Nuclear	Peroxisome Proliferator	nr_ppar_gamma	Inactive	0.95

Classification	Target	Shorthand	Prediction	Probability
receptor signalling pathways	Activated Receptor Gamma (PPAR-Gamma)			
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.82
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.57
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.88
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.63
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Active	0.60
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.97
Metabolism	Cytochrome CYP2C9	CYP2C9	Inactive	0.88
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.84
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.90

Result:

Compound	Drug-likeness Score	Predicted LD50	Toxicity Class	Major Toxicity Observation	Interpretation
Kaempferol	0.50	3919 mg/kg	Class 5	Low toxicity profile with inactive hepatotoxicity and cardiotoxicity	Kaempferol showed good drug-likeness with lower toxicity, indicating suitability as a potential therapeutic phytoconstituent.
Luteolin	0.38	3919 mg/kg	Class 5	Mild nephrotoxicity and activation of CYP enzymes observed	Luteolin demonstrated acceptable safety profile and moderate drug-likeness, suggesting possible pharmacological potential with careful

					dose consideration.
Gallic Acid	0.29	2000 mg/kg	Class 4	Mild carcinogenicity and nephrotoxicity prediction observed	Gallic acid exhibited moderate toxicity compared to other compounds but still possessed considerable therapeutic potential.
Quercetin	0.75	Safe range predicted	Low toxicity	Strong antioxidant and low toxic effects	Quercetin showed the highest drug-likeness score among studied compounds indicating excellent pharmacological potential.
Daucosterol	0.52	Moderate toxicity	Class 5	Minimal toxic effects predicted	Daucosterol demonstrated acceptable safety and moderate drug-likeness characteristics.

Overall Interpretation:
The overall network pharmacology and toxicity analysis demonstrated that the phytoconstituents present in Punica granatum leaves possess significant therapeutic potential with comparatively low toxicity profiles. Compounds such as quercetin, kaempferol, luteolin, and gallic acid showed favorable drug-likeness properties and acceptable safety parameters. The ADME and toxicity prediction studies suggested that most compounds may be suitable for further pharmacological and drug development research. The results support the traditional medicinal importance of *Punica granatum* and validate its multi-target therapeutic applications through network pharmacology approaches.[26]

Network pharmacology Interpretation:
Network pharmacology analysis of Punica granatum leaves indicated that the bioactive phytoconstituents such as flavonoids, tannins, and phenolic compounds may interact with multiple target proteins and biological pathways associated with inflammation, oxidative stress, diabetes, and cancer. The compound-target interaction studies suggested that the therapeutic effects of *Punica granatum* are due to synergistic action of multiple phytochemicals rather than a single active constituent.Pathway enrichment analysis demonstrated the involvement of various signaling pathways related to antioxidant defense, inflammatory response, and cellular protection. These findings support the traditional medicinal uses of *Punica granatum* leaves and highlight their potential importance in multi-target drug discovery through network pharmacology approaches.[27]

Conclusion:
The present study on Punica granatum leaves demonstrated the presence of important phytoconstituents such as flavonoids, tannins, alkaloids, glycosides, phenolic compounds, and terpenoids. These bioactive compounds are responsible for various pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective effects. Preliminary phytochemical screening confirmed the medicinal potential of the plant and supported its traditional therapeutic uses.Network pharmacology analysis suggested that the phytochemicals present in *Punica granatum*

leaves may act on multiple target proteins and biological pathways involved in different diseases. The study highlights the importance of multi-component and multi-target mechanisms of herbal medicines. Therefore, *Punica granatum* leaves can be considered a promising natural source for future pharmacological research and development of novel therapeutic agents.

Future Scope

- Further isolation and characterization of bioactive phytoconstituents from *Punica granatum* leaves.
- Detailed molecular docking and in silico studies for identification of target proteins.
- Advanced network pharmacology studies to understand multi-target therapeutic mechanisms.
- Development of herbal formulations using *Punica granatum* leaf extract.
- Clinical studies to evaluate safety and therapeutic efficacy in humans.
- Toxicological studies for determination of safe dosage and long-term effects.
- Investigation of anticancer and antidiabetic potential through experimental models.
- Exploration of antioxidant and anti-inflammatory activities for chronic disease management.
- Development of novel plant-based drugs from *Punica granatum* leaves.
- Use of nanotechnology and modern drug delivery systems for improved therapeutic effectiveness.

REFERENCES:

1. Jurenka JS. Th
2. erapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alternative Medicine Review*. 2008;13(2):128-144.
3. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*. 2007;109(2):177-206.
4. Viuda-Martos M, Fernández-López J, Pérez-Álvarez JA. Pomegranate and its many functional components as related to human health: A review. *Comprehensive Reviews in Food Science and Food Safety*. 2010;9(6):635-654.
5. Elfalleh W, Hannachi H, Tlili N, Yahia Y, Nasri N, Ferchichi A. Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*. 2012;6(32):4724-4730.
6. Holland D, Hatib K, Bar-Ya'akov I. Pomegranate: Botany, horticulture, breeding. *Horticultural Reviews*. 2009;35:127-191.
7. Ismail T, Sestili P, Akhtar S. Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects. *Journal of Ethnopharmacology*. 2012;143(2):397-405.
8. Hopkins AL. Network pharmacology: The next paradigm in drug discovery. *Nature Chemical Biology*. 2008;4(11):682-690.
9. Li S, Zhang B. Traditional Chinese medicine network pharmacology: Theory and methodology. *Chinese Journal of Natural Medicines*. 2013;11(2):110-120.
10. Hopkins AL. Network pharmacology: The next paradigm in drug discovery. *Nature Chemical Biology*. 2008;4(11):682-690.
11. Li S, Zhang B. Traditional Chinese medicine network pharmacology: Theory and methodology. *Chinese Journal of Natural Medicines*. 2013;11(2):110-120.
12. Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HHHW. Network pharmacology: Curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*. 2022;43(2):136-150.
13. .Chandran U, Mehendale N, Tillu G, Patwardhan B. Network pharmacology: An emerging technique for natural product drug discovery and scientific research on Ayurveda. *Pharmacological Research*. 2017;115:110-120.
14. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*. 2007;109(2):177-206.

15. Holland D, Hatib K, Bar-Ya'akov I. Pomegranate: Botany, horticulture, breeding. *Horticultural Reviews*. 2009;35:127-191.
16. Kumar A, et al. Pharmacognostic and phytochemical evaluation of *Punica granatum* leaves. *Asian Pacific Journal of Tropical Biomedicine*. 2012;2(2):S941-S946.
17. Jurenka JS. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alternative Medicine Review*. 2008;13(2):128-144.
18. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*. 2007;109(2):177-206.