

# Understanding the Mechanism of Shilajit by Using Network Pharmacology Approach

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## Guide

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# Introduction: What is Shilajit?

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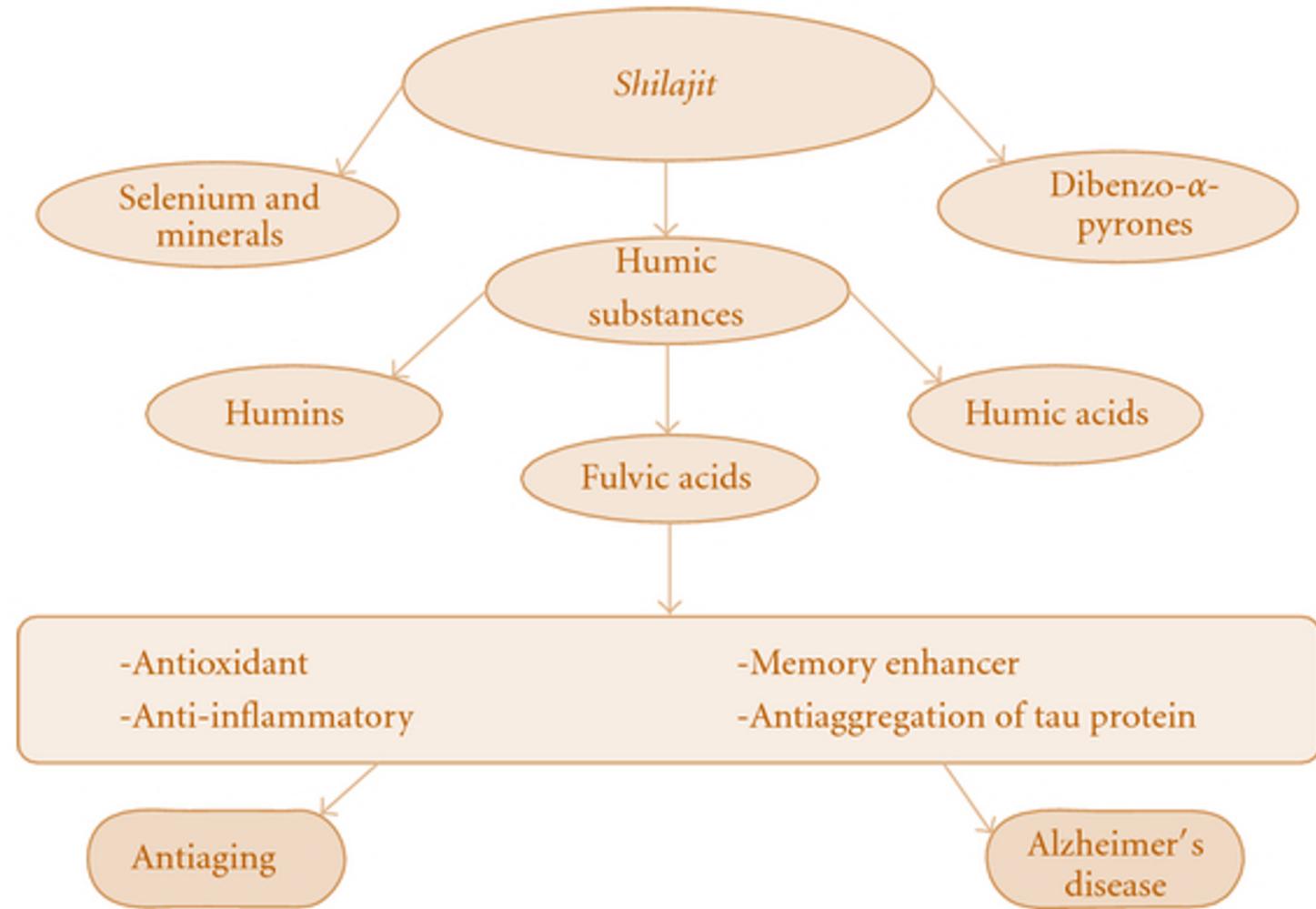
**Shilajit** is a blackish-brown exudate from high mountain rocks and is produced over a couple of mineral years from organic matters and plants that have been trapped between the layers of rocks in mountains in a few regions. With time, the pressure from the weight of the mountains causes the materials to be transformed into a rich mineral mass which then oozes out of the rocks.



# Chemical composition

The chemical composition of Shilajit is very complex. It is a phyto-complex which contains:

- **Humic Substances (HS) –**
  - Fulvic Acids
  - Humins
  - Humic Acids
- It also contains **selenium and other minerals** in traces.
- Shilajit also contains small amount of **dibenzo- $\alpha$ -pyrones**.



# **Medicinal Properties of Shilajit**

**Antilithiatic  
(stops  
formation of  
stones in  
kidneys)**

**Cardio-  
protective**

**Anti-  
diabetic**

**Anti-  
inflammatory**

**Rejuvenative  
agent**

**Aphrodisiac**

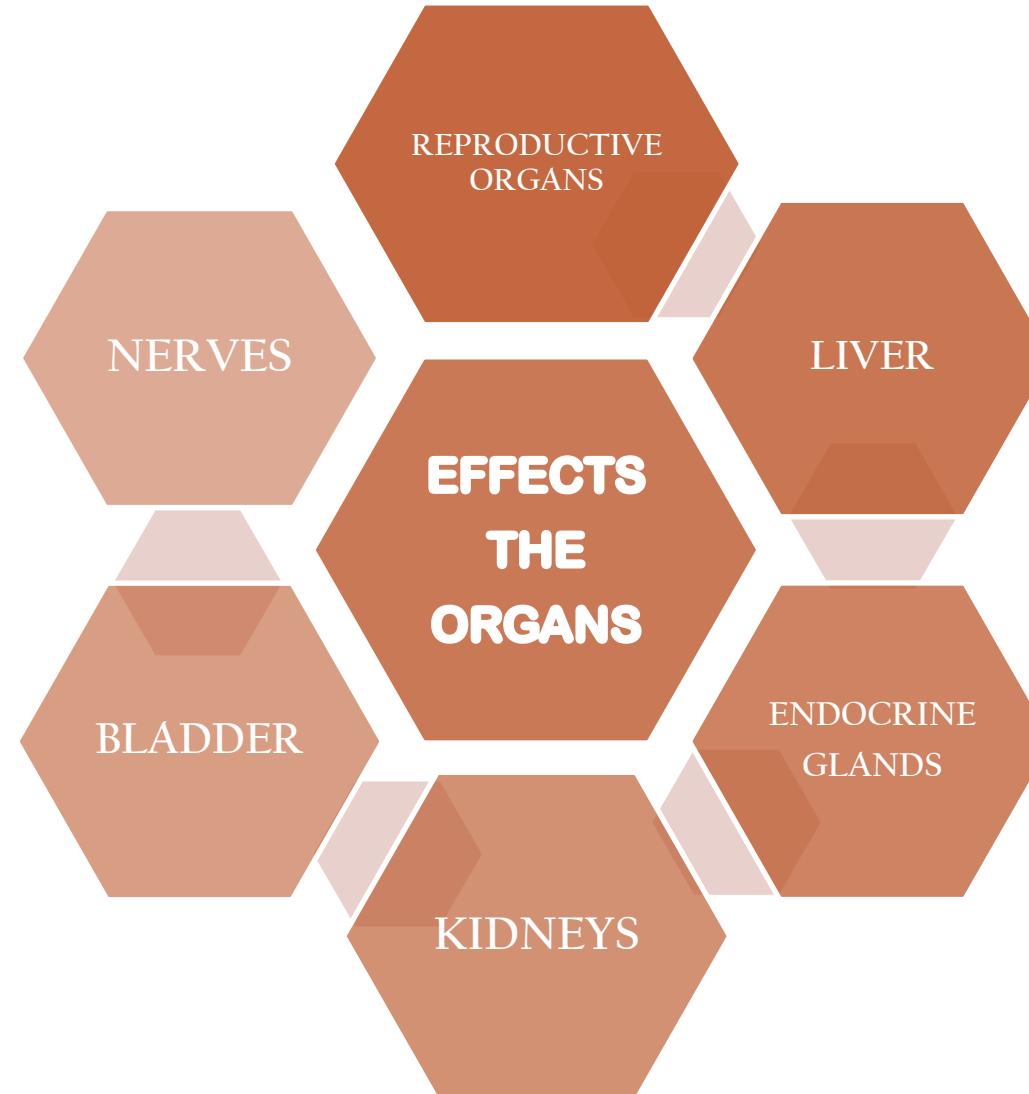
**Nervine  
Tonic**

**Anti-  
obesity**

**Anxiolytic**

# Therapeutic Properties

According to traditional Indian knowledge, Shilajit is generally useful as health tonic or traditional nutritional supplement. In addition to its supplementary uses, it is also beneficial for treating a variety of diseases. In ayurveda, it is widely used for its therapeutic value.



# Hypothesis

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Pharmacological network of *Shilajit* will depict the interaction of bio-actives with molecular targets of cancer.

# Objectives

1. To find the bio-actives of *Shilajit*.
2. To predict the targets of the components of *Shilajit*.
3. To construct a pharmacology network of the mechanism of formulation of *Shilajit*.
4. To validate the findings.

# NETWORK PHARMACOLOGY APPROACH

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- Network pharmacology is an emerging discipline useful in formulation discovery, which integrates recent advances in omics technologies and systems biology through computational biology.
- Recently, this technique has been applied in understanding the underlying mechanism of Ayurveda formulations. In this study, the network pharmacology approach will be used as a tool to understand the pharmacodynamics of *Shilajit*.
- Network pharmacology targets biological networks and analyzes the links among drugs, targets, and diseases in those networks.
- This will be done using the software programs Cytoscape.

**The general analysis process of Network Pharmacology can be described as follows:**

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1. Interaction information retrieval from databases.
2. Network construction.
3. Knowledge discovery based on network models.

# Binding Database

It is a web-based free database that covers protein interactions with small drug-like molecules. It searches for the exact or similar compounds in the database and retrieves the target information of those compounds.

The screenshot shows the main page of the BindingDB website. At the top right, the title "The Binding Database" is displayed. Below it is a navigation bar with links: Home, Info, Download, About us, Email us, Contribute data, and Web Services. On the left side, there is a sidebar with various search and browse options. A red arrow points from the text "Find My Compound's Targets" in the sidebar down to the "Find My Compound's Targets" link in the footer. The main content area contains several sections: "Simple Search" (with a search input field and "Go" button), "Advanced Search" (described as combining multiple search criteria), "Messages" (noting data extraction from US Patents), "Patent Curation by BindingDB" (listing statistics: Patents: 2,732, Binding measurements: 353,702, Compounds: 207,422, Target proteins: 1,561, Assays: 3,947, and Average Number of Targets per Patent: 1.91), and a section on journal curation (listing journals like ACS Chemical Biology, ACS BioChemistry, Bioorganic Chemistry, BMC Chemical Biology, etc.).

**myBDB logout**

**Search and Browse**

Target

Sequence

Name &

K<sub>i</sub> IC<sub>50</sub> Kd EC<sub>50</sub>

Rate constants

ΔG° ΔH° -TΔS°

pH (Enzymatic Assay)

pH (ITC)

Substrate or Competitor

Compound Mol. Wt.

Chemical Structure

Pathways

Source Organism

Number of Compounds

Monomer List in csv

Het List in SDF

Compound

FDA Drugs

Important Compounds

Chemical Structure

Name

SMILES

Number of Data / Targets

Special tools

3D Structure Series

**Find My Compound's Targets**

**Find Compounds for My Targets**

**Simple Search**

Article Titles, Authors, Assays, Compound Names, Target Names

Use ? for single-letter wild-card or \* for general wild-card.  
For example, "adeny\*" or "adeny?". Query cannot start with wild card.

**Advanced Search**

Combine multiple search criteria, such as chemical structures, target names, and numerical affinities; restrict searches by data source, such as BindingDB, ChEMBL, PubChem, and Patents.

**Messages**

From 11/2017 to 10/2018, BindingDB curators extracted over 48,000 data (27,500 compounds and 400 targets) from US Patents! (November 09, 2018)

**Patent Curation by BindingDB**

Patents: 2,732  
Binding measurements: 353,702  
Compounds: 207,422  
Target proteins: 1,561  
Assays: 3,947  
Average Number of Targets per Patent: 1.91

Continually curates a set of journals not covered by other public databases. As of April 2019, the status of our current curation effort is as follows:

ACS Chemical Biology 2006-2017 (vol 1-12)  
ACS BioChemistry 1965-2017 (vol 4-56)  
Bioorganic Chemistry 1990-2017 (vol 18-73)  
BMC Chemical Biology 2001-2010 (vol 1-10)  
Biochemistry 1990-2017 (vol 1-17)

## BindingDB News

November 2017. If you are interested in preparing a multi-targeted compound collection, you may be interested in our new download. This file lists all purchasable compounds for all Targets in BindingDB, with an affinity better than 10 micromolar, and includes catalog information. See "Purchasable Compounds by Target" on our Download page.

September 2017. The Advanced Search page has been simplified and made more unified with other BindingDB pages.

June 2017. We are pleased to report that the NIH has renewed its support for BindingDB. Thanks to all who filled out our survey and provided supporting messages!

June 2017. Drug Design Data Resource (D3R) datasets have been integrated into BindingDB and are also available here:  
<https://www.bindingdb.org/bind/ByD3R.jsp>

# DrugBank

The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information.

The screenshot shows the DrugBank website's search interface. At the top, there is a navigation bar with links for Browse, Search, Downloads, About, Help, Blog, and Contact Us. Below the navigation bar is a search bar with the placeholder "WHAT ARE YOU LOOKING FOR?". In the search bar, the word "Tylenol" is typed. To the right of the search bar is a magnifying glass icon. Below the search bar, there are four buttons: "Drugs" (highlighted in pink), "Targets", "Pathways", and "Indications".



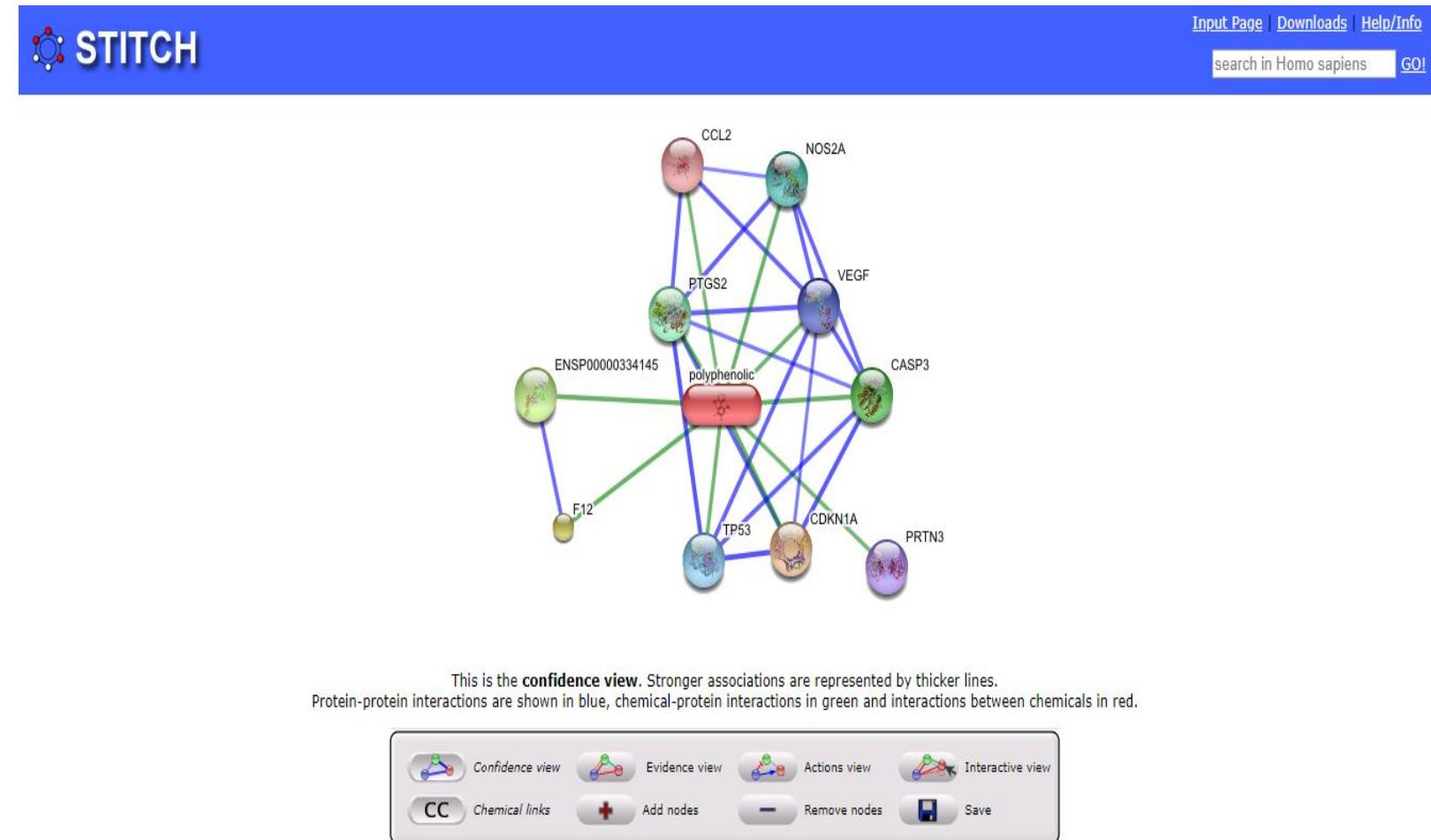
The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information.

The latest release of DrugBank (version 5.1.3, released 2019-04-02) contains 12,458 drug entries including 2,581 approved small molecule drugs, 1,286 approved biotech (protein/peptide) drugs, 130 nutraceuticals and over 6,103 experimental drugs. Additionally, 5,169 non-redundant protein (i.e. drug target/enzyme/transporter/carrier) sequences are linked to these drug entries. Each DrugCard entry

# STITCH:

## Chemical-Protein Interactions

STITCH is a resource to explore known and predicted interactions of chemicals and proteins. Chemicals are linked to other chemicals and proteins by evidence derived from experiments, databases and the literature.



# Therapeutic Target Database

The targets of the bioactives are searched in the TTD for their association with any disease or indication.

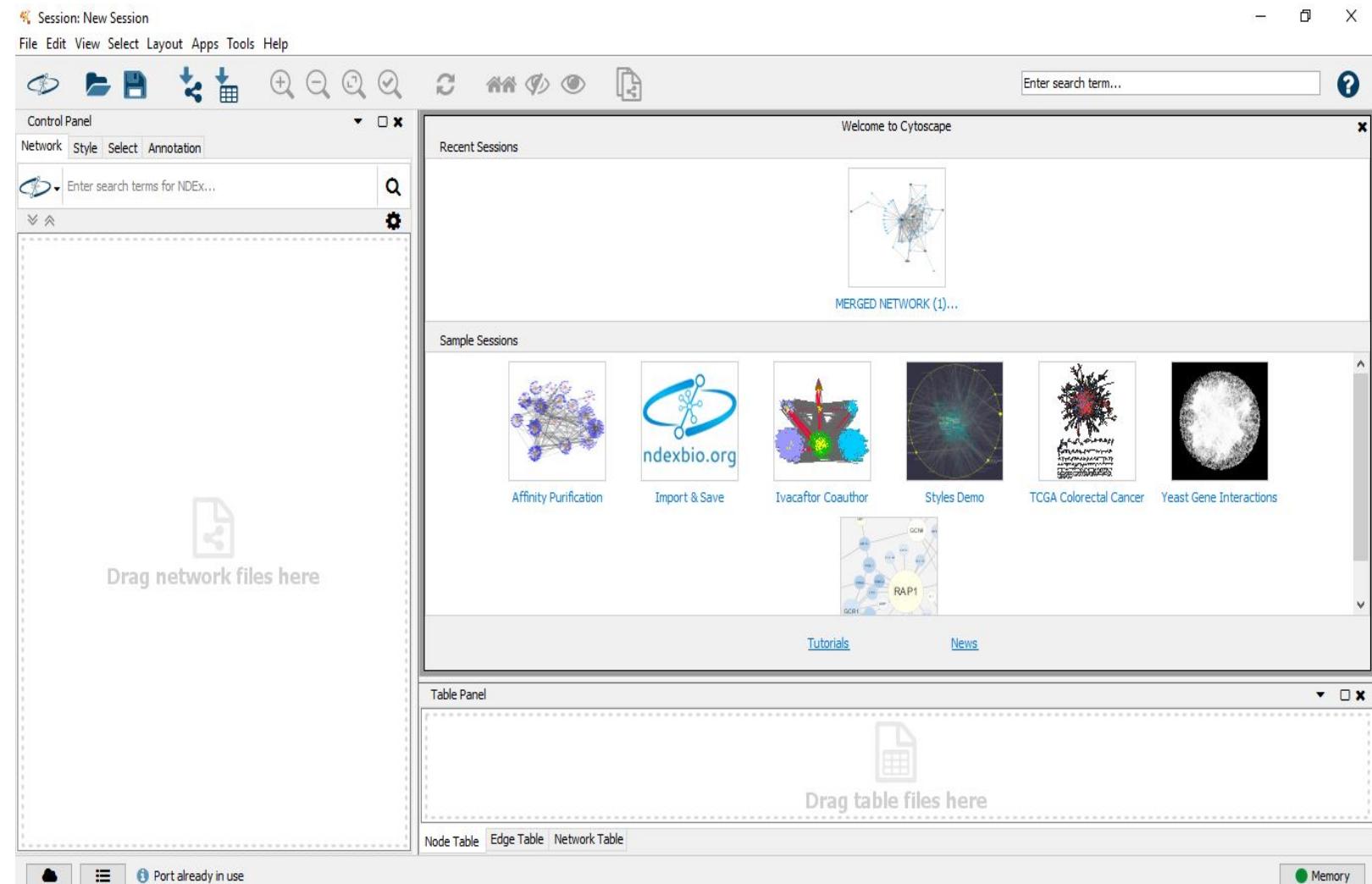
The screenshot shows the Therapeutic Target Database (TTD) interface. At the top, there is a logo for TTD (a stylized sunburst icon) and BIDD (Bioinformatics and Drug Design group) with a blue and yellow circular graphic. The main navigation menu includes Home, Advanced Search, Patient Data, Targets / Drugs Group, Model & Study Data, and Download. Below the menu, a section titled "Target Information" is displayed. A table under "Target General Information" lists details for Target ID T76685, including Former ID TTDS00334, Target Name Cannabinoid receptor 1, Gene Name CNR1, Synonyms CANN6; CB-R; CB1; Cannabinoid CB1 receptor; CNR1, and Target Type Successful. Below this table, a list of associated diseases and indications is shown: Anorexia [ICD9: 307.1; ICD10: F50.0-F50.1], Central nervous system disease [ICD10: G00-G99], Cerebrovascular ischaemia [ICD9: 434.91; ICD10: I61-I63], Chemotherapy-induced nausea [ICD9: 787, 787.0; ICD10: R11], Diabetes; Obesity [ICD9: 250, 278; ICD10: E08-E13, E66], and Drug abuse [ICD9: 303-304; ICD10: F10-F19].

Target General Information	
Target ID	T76685
Former ID	TTDS00334
Target Name	Cannabinoid receptor 1
Gene Name	CNR1
Synonyms	CANN6; CB-R; CB1; Cannabinoid CB1 receptor; CNR1
Target Type	Successful
	Anorexia [ICD9: 307.1; ICD10: F50.0-F50.1]
	Central nervous system disease [ICD10: G00-G99]
	Cerebrovascular ischaemia [ICD9: 434.91; ICD10: I61-I63]
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	Diabetes; Obesity [ICD9: 250, 278; ICD10: E08-E13, E66]
	Drug abuse [ICD9: 303-304; ICD10: F10-F19]

# Cytoscape

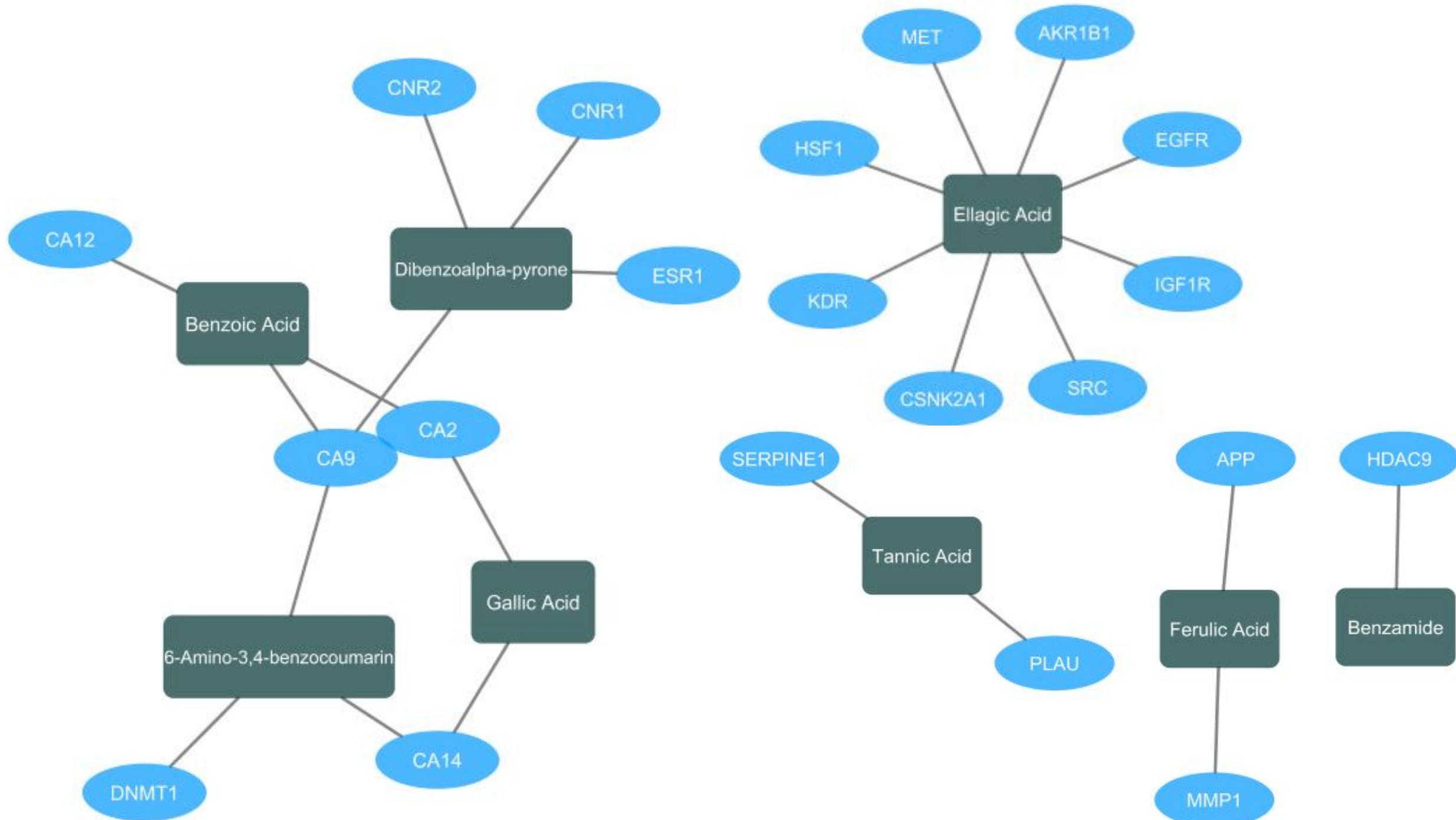
Cytoscape is a free, open-source, visual interface for importing, visually exploring, and analyzing molecular interaction networks. It provides 2D representations.

There are also various plug-ins for Cytoscape, which allow users to perform many analysis such as graph-based studies, network inference, functional enrichment studies, etc.



# Targets found through Binding Database

	A	B	C
1	#node1	#node2	
2	CNR2	Dibenzoalpha-pyrone	
3	CNR1	Dibenzoalpha-pyrone	
4	CA9	Dibenzoalpha-pyrone	
5	ESR1	Dibenzoalpha-pyrone	
6	CA9	6-Amino-3,4-benzocoumarin	
7	CA14	6-Amino-3,4-benzocoumarin	
8	DNMT1	6-Amino-3,4-benzocoumarin	
9	HDAC9	Benzamide	
10	CA12	Benzoic Acid	
11	CA2	Benzoic Acid	
12	CA9	Benzoic Acid	
13	AKR1B1	Ellagic Acid	
14	CSNK2A1	Ellagic Acid	
15	EGFR	Ellagic Acid	
16	HSF1	Ellagic Acid	
17	MET	Ellagic Acid	
18	IGF1R	Ellagic Acid	
19	SRC	Ellagic Acid	
20	KDR	Ellagic Acid	
21	APP	Ferulic Acid	
22	MMP1	Ferulic Acid	
23	CA2	Gallic Acid	
24	CA14	Gallic Acid	
25	SERPINE1	Tannic Acid	
26	PLAU	Tannic Acid	
27			



# STRING

(Search Tool for the Retrieval of  
Interacting Genes/Proteins)

It is a biological database and web resource of known and predicted protein - protein interactions. The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections.

Version: 11.0 [LOGIN](#) | [REGISTER](#)

 STRING

SEARCH

Single Protein by Name / Identifier

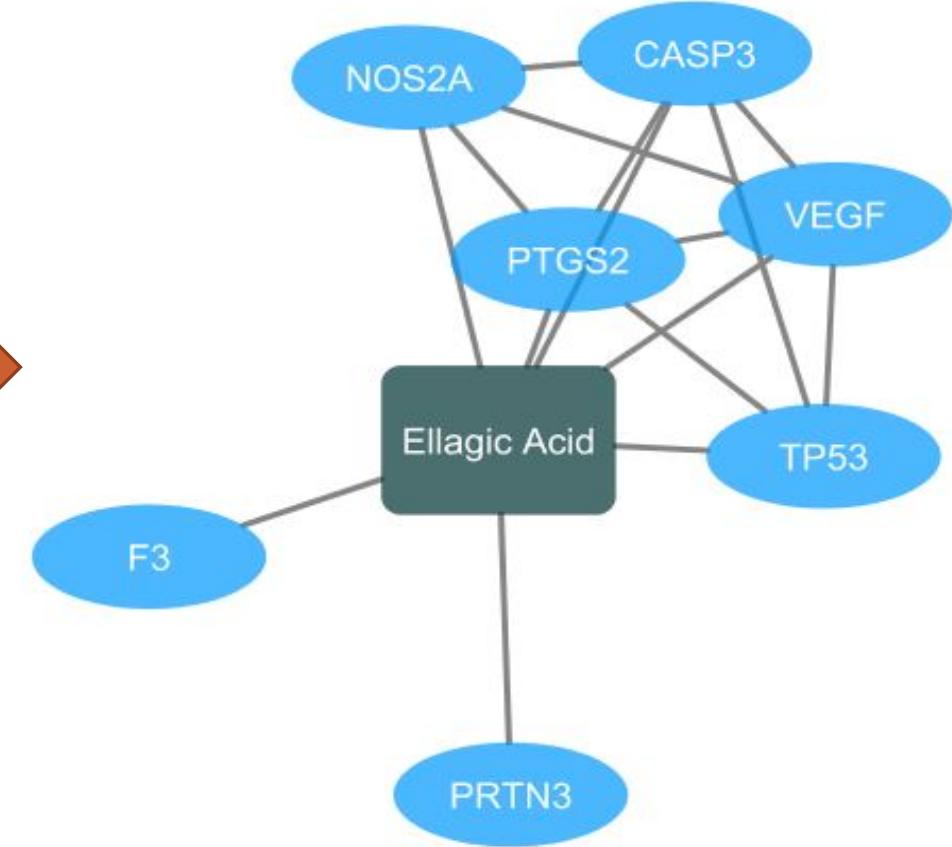
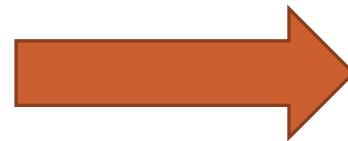
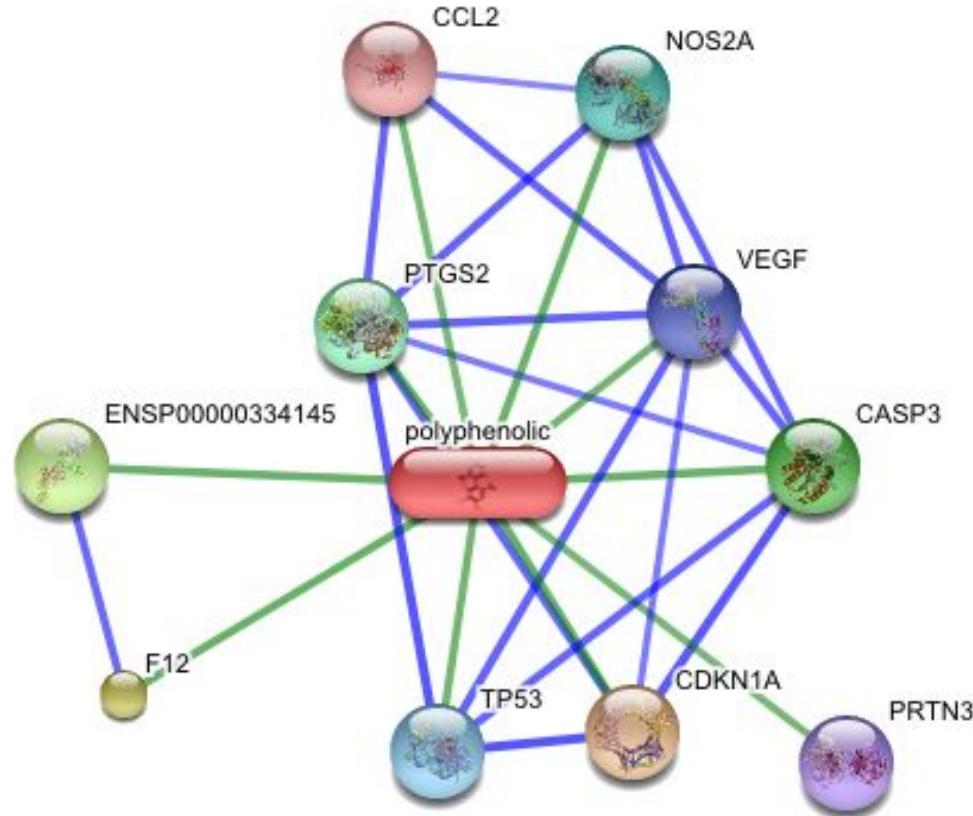
Protein Name: (examples: #1 #2 #3)

Organism:

SEARCH

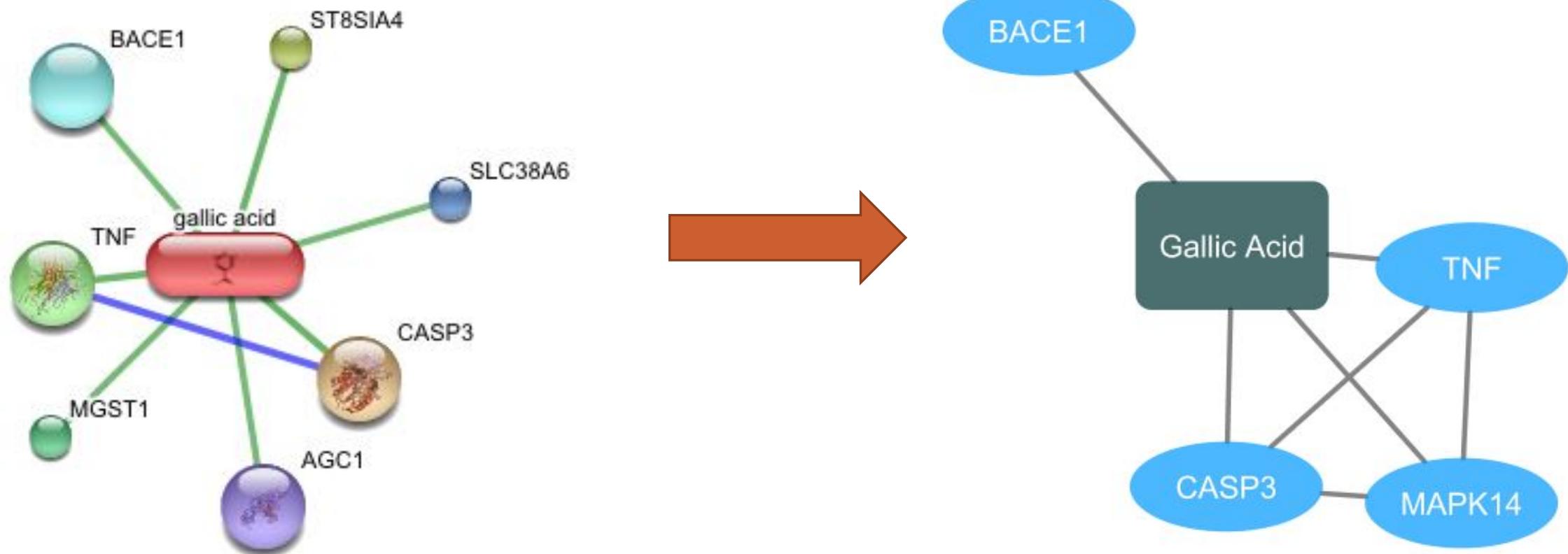
- [Protein by name](#)
- [Protein by sequence](#)
- [Multiple proteins](#)
- [Multiple sequences](#)
- [Proteins with Values/Ranks New](#)
- [Organisms](#)
- [Protein families \("COGs"\)](#)
- [Examples](#)
- [Random entry](#)

# Ellagic Acid Network



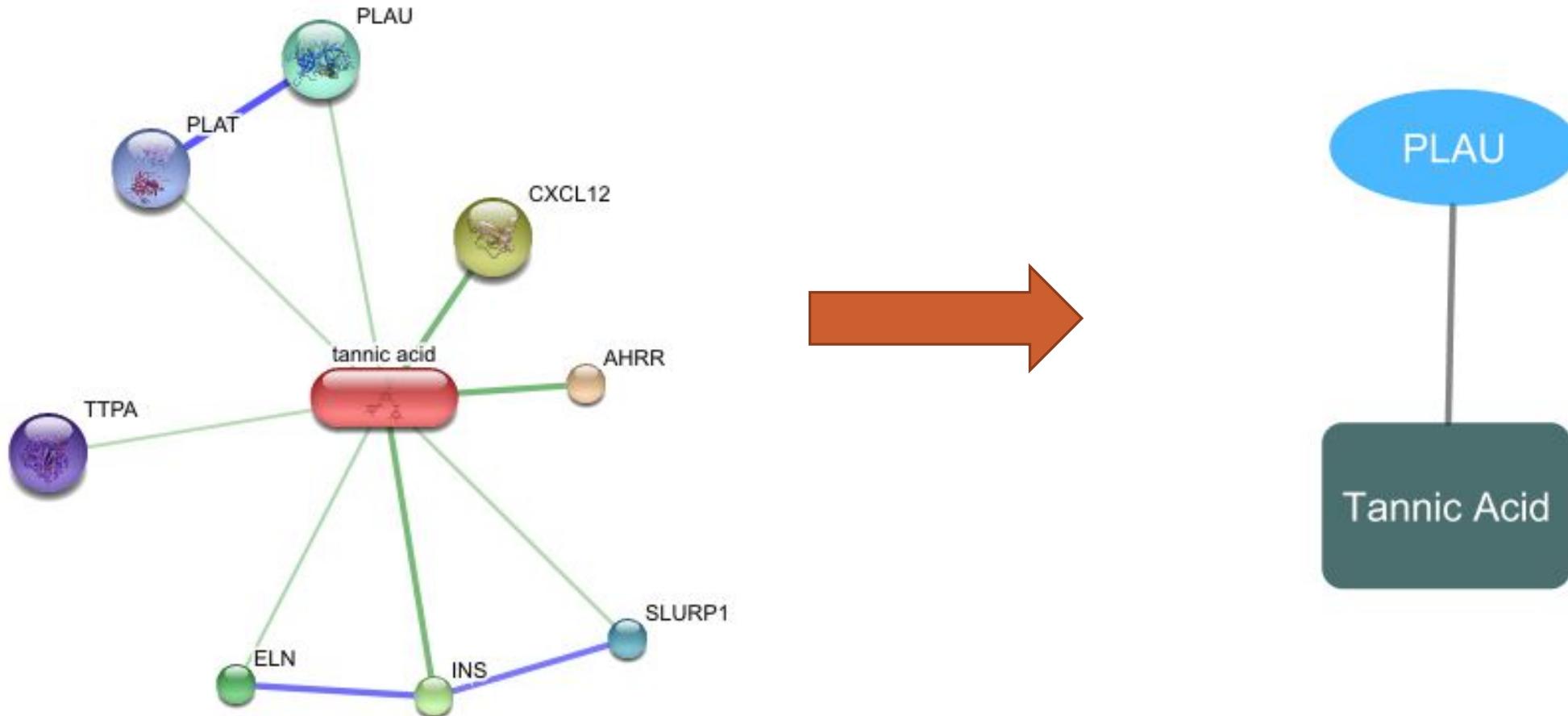
# Gallic Acid Network

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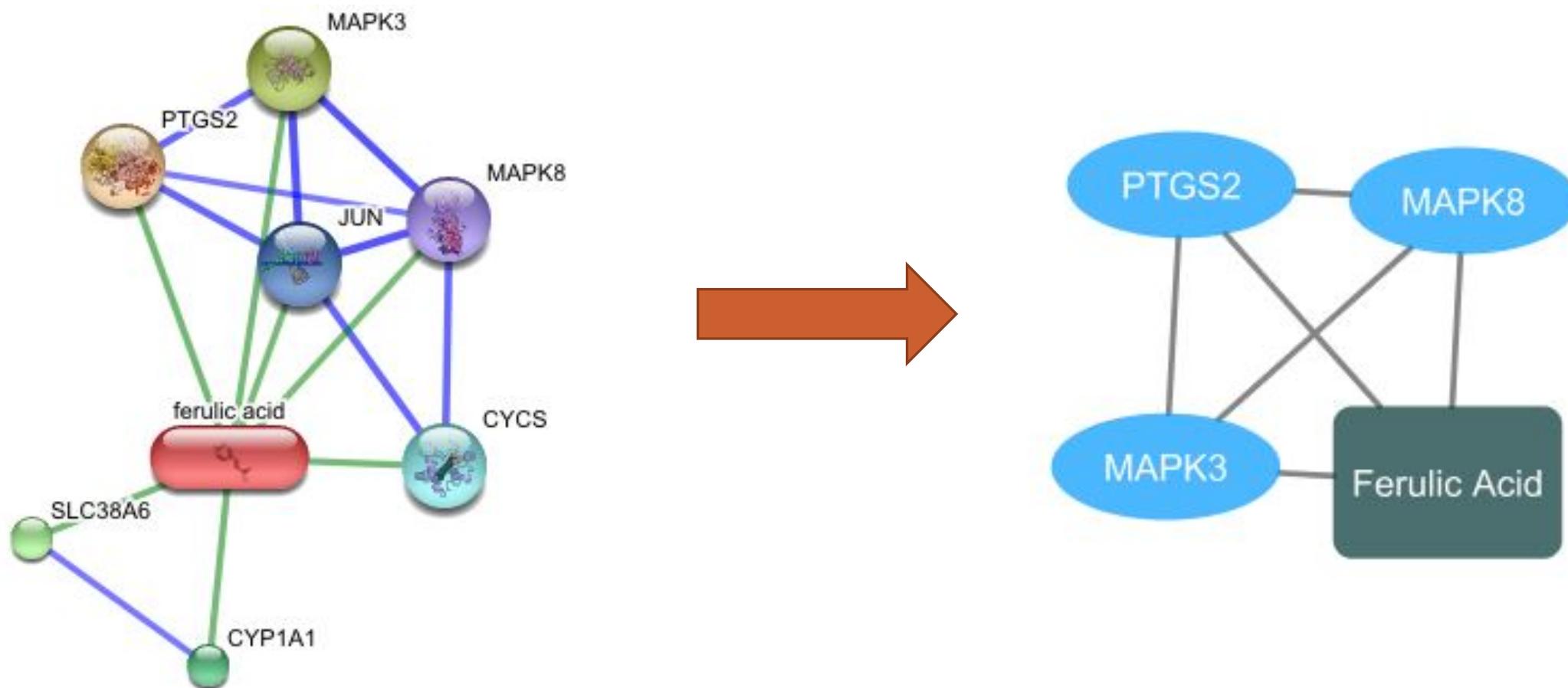
# Tannic Acid

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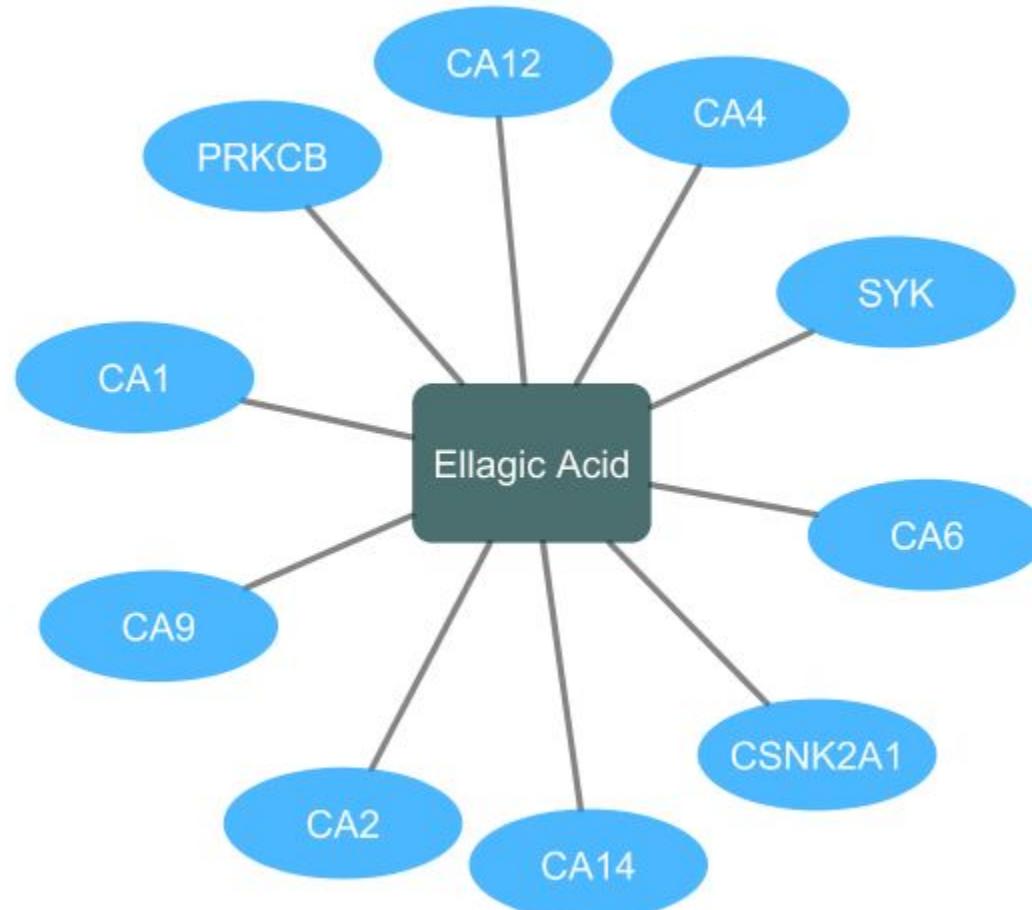
# Ferulic Acid

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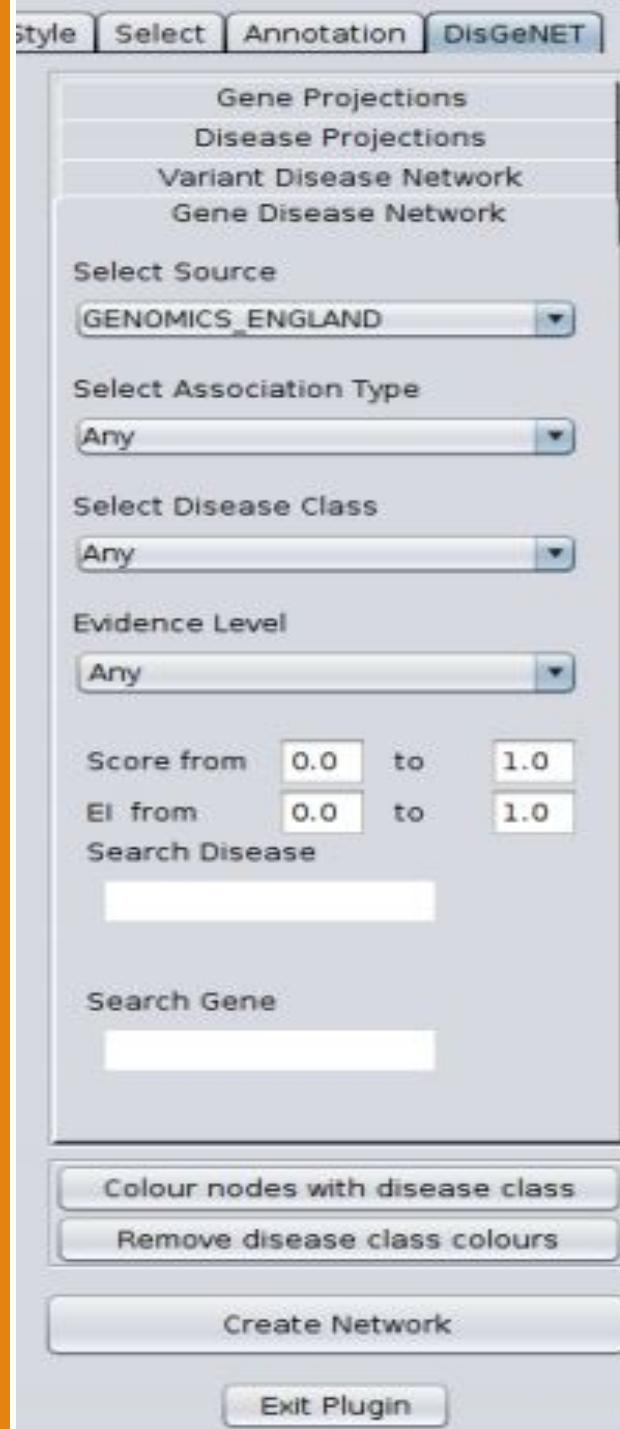
# Ellagic Acid Network (DrugBank)

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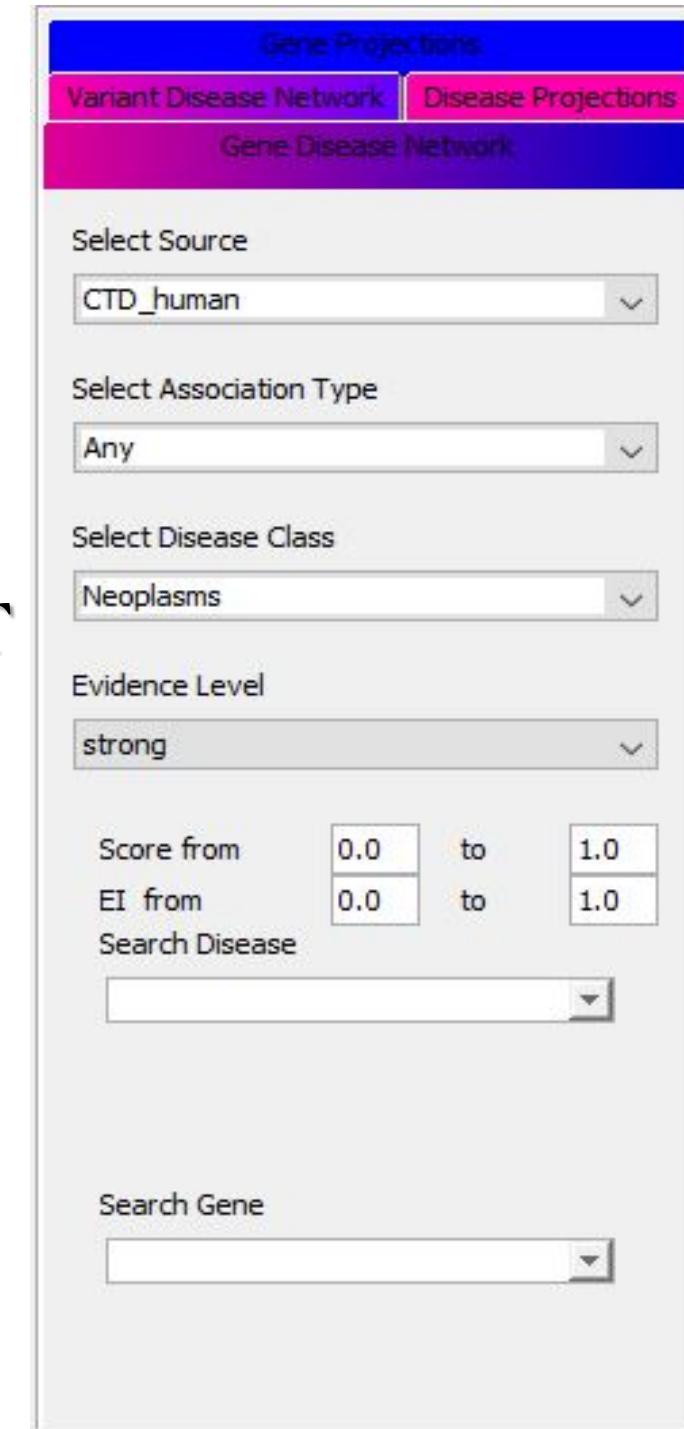


# DisGeNET

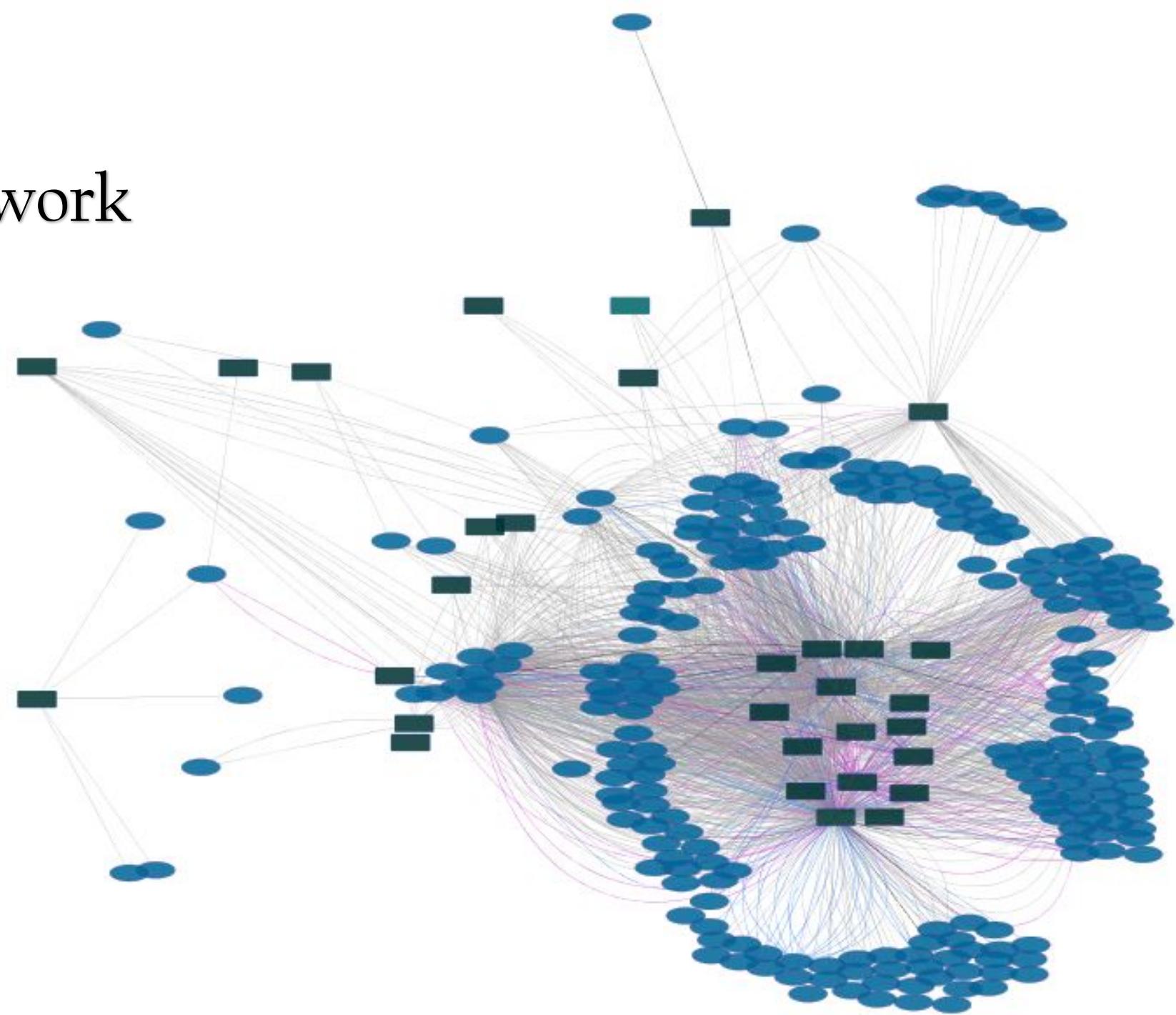
The DisGeNET Cytoscape app is designed to visualize, query and analyze a network representation of the gene-disease and the variant - disease associations contained in DisGeNET.

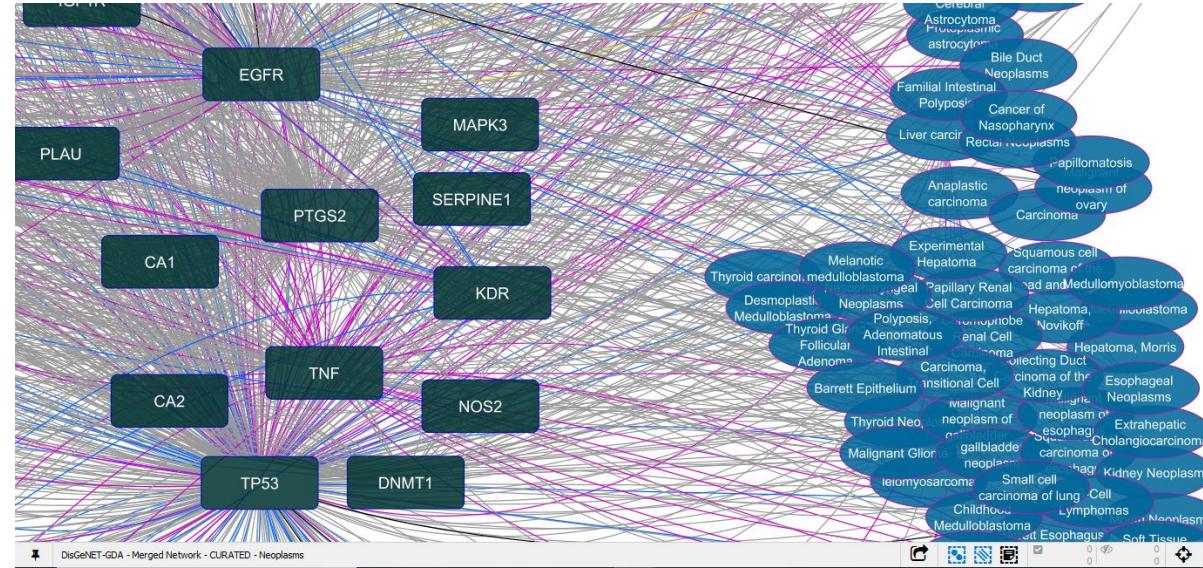
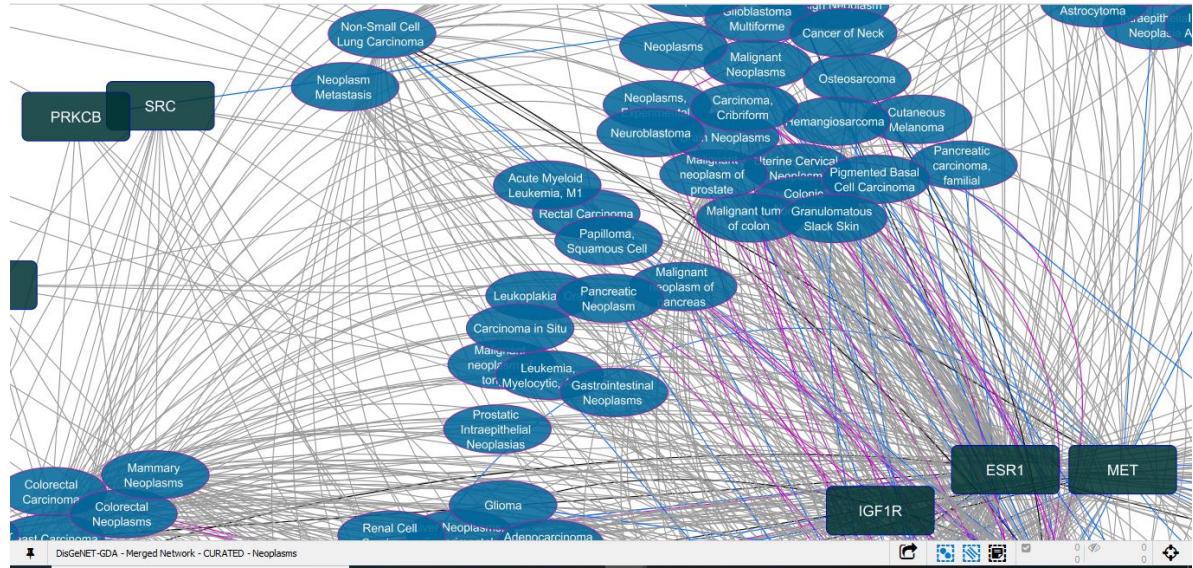


# DisGeNET Control Panel



# Shilajit-cancer Network





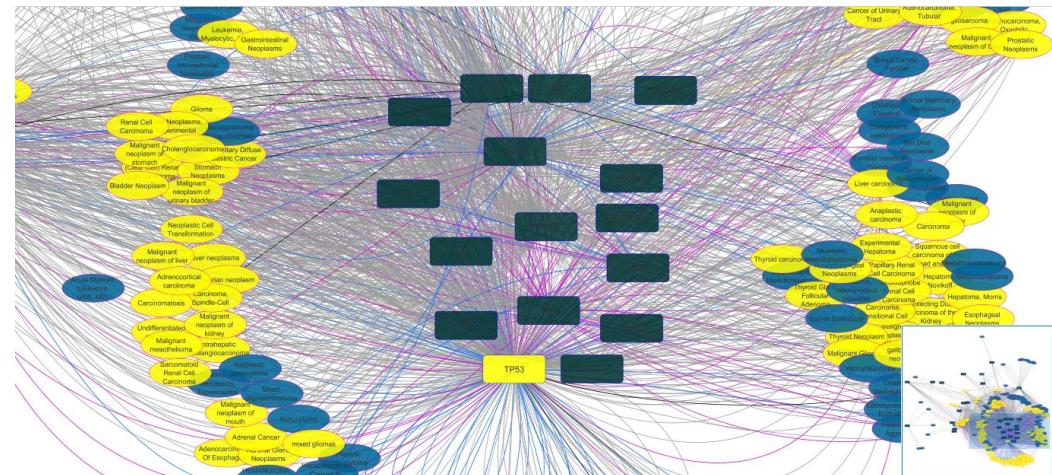
On zooming into the Shilajit-cancer network, one can notice all the different diseases that the targets are associated to. For each of the targets, this information has been collected from various different literature sources. In figures 36 and 37, the various diseases associated to the targets SRC, PRKCB, EGFR, CA2, TP53, DNMT1, NOS2, MAPK3, PTGS2, etc. are shown.

In order to see which bioactive had the most associations with neoplasm diseases, each bioactive node was selected, and the number of diseases associated to it was counted. This was noted down in the form of a table.

## NETWORK NODES TABLE

styleName	nodeType	geneName	geneId	diseaseName	diseaseId	diseaseClassName	diseaseClass
HSF1	gene	HSF1	3297				
F3	gene	F3	2152				
ESR1	gene	ESR1	2099				
EGFR	gene	EGFR	1956				
DNMT1	gene	DNMT1	1786				
CSNK2A1	gene	CSNK2A1	1457				
CNR2	gene	CNR2	1269				
CNR1	gene	CNR1	1268				
CA9	gene	CA9	768				
CA2	gene	CA2	760				
CA12	gene	CA12	771				
CA1	gene	CA1	759				
AKR1B1	gene	AKR1B1	231				
Mammary Carcinoma, Animal	disease						
Animal Mammary Neoplasms	disease						
Li-Fraumeni-Like Syndrome	disease						
Li-Fraumeni Syndrome	disease						
LI-FRAUMENI SYNDROME 1	disease						
HEMANGIOMA, CAPILLARY INFAR...	disease						
Polyposis, Adenomatous Intestinal	disease						
Familial Intestinal Polyposis	disease						
Adenomatous Polyposis Coli	disease						
Pancreatic carcinoma, familial	disease						
Pancreatic carcinoma	disease						
Pancreatic Neoplasm	disease						
Malignant neoplasm of pancreas	disease						
Carcinoma, Pancreatic Ductal	disease						
gallbladder neoplasm	disease						
Stomach Neoplasm	disease						
Squamous cell carcinoma of esop...	disease						
Rectal Neoplasms	disease						
Rectal Carcinoma	disease						
Malignant tumor of colon	disease						
Malignant neoplasm of stomach	disease						
Malignant neoplasm of liver	disease						
Malignant neoplasm of gallbladder	disease						

## DISEASES ASSOCIATED TO TP53



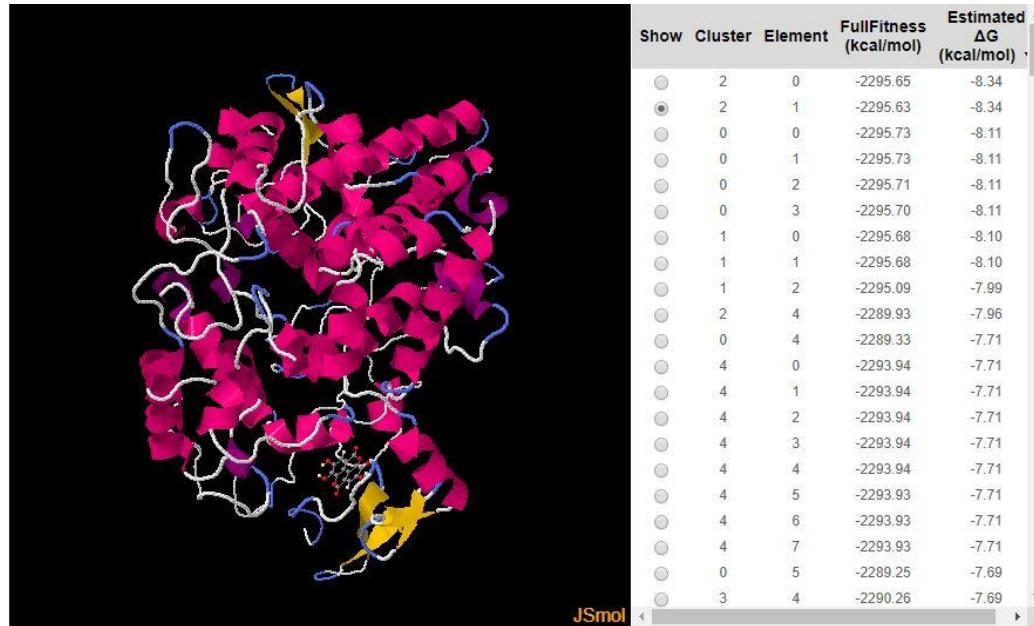
From the table, it was observed that ellagic acid had the most associations. This is because of the targets TP53, PTGS2, EGFR, MET, etc. The sum total of all the interactions of ellagic acid's targets with cancer related diseases is the highest compared to the others.

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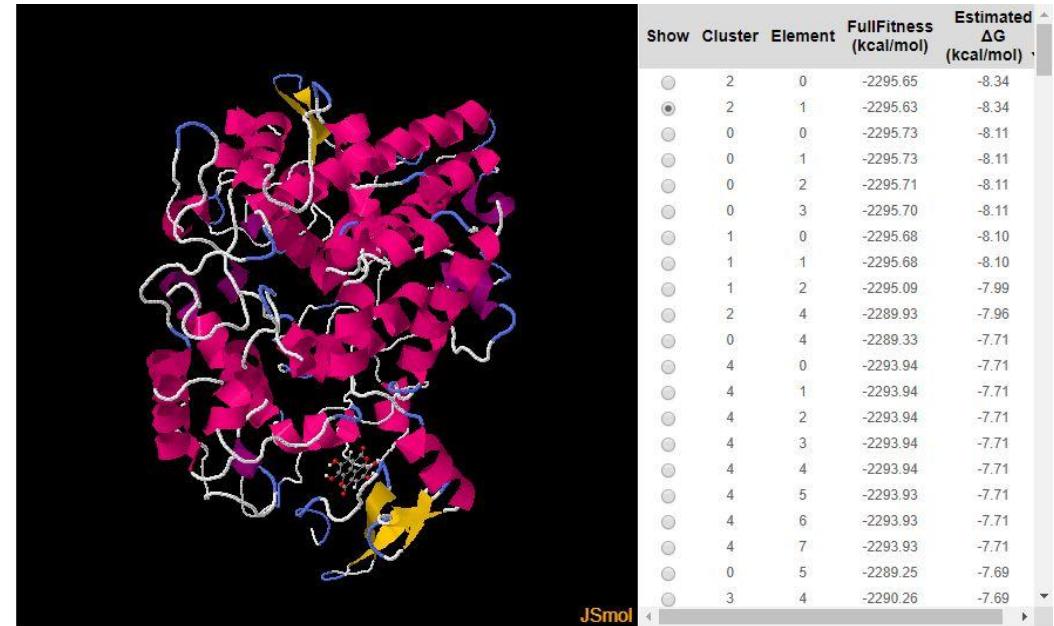
geneName	No. of Interactions	Bio-active
TP53	146	Ellagic Acid
PTGS2	90	Ellagic Acid + Ferulic Acid
EGFR	87	Ellagic Acid
MET	67	Ellagic Acid
ESR1	57	Dibenzoalpha-pyrone
VEGFA	51	Ellagic Acid
TNF	47	Gallic Acid
DNMT1	31	6-Amino-3,4-benzocoumarin
KDR	27	Ellagic Acid
MAPK3	25	Ferulic Acid
PLAU	19	Tannic Acid
NOS2	14	Ellagic Acid
SRC	13	Ellagic Acid
MMP1	12	Ferulic Acid
IGF1R	11	Ellagic Acid
CA2	11	Ellagic Acid + Benzoic Acid
CA1	10	Ellagic Acid
CNR2	9	Dibenzoalpha-pyrone
PRKCB	8	Ellagic Acid
MAPK8	8	Ferulic Acid
SERPINE1	7	Tannic Acid
MAPK14	6	Gallic Acid
F3	5	Ellagic Acid

# Validation by Docking

DOCKING ELLAGIC ACID WITH PTGS2



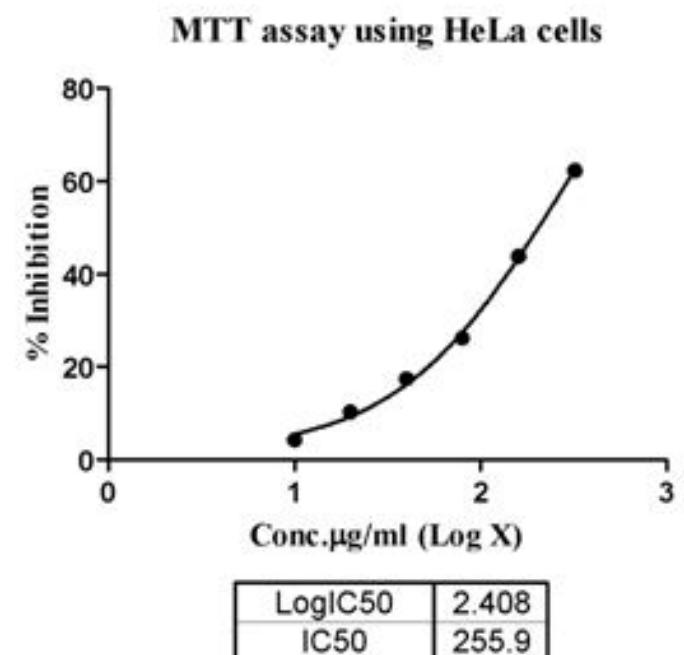
DOCKING ELLAGIC ACID WITH EGFR



The docking studies of ellagic acid with TP53 were not done due to the sparse literature information present on its 3D structure.

# Validation Using Cancer Cell Line

Compound name	Conc. µg/ml	OD at 590nm	% Inhibition	IC50 µg/ml
Control	0	0.752	0.00	
<i>Shilajit</i>	10	0.719	4.32	255.90
	20	0.675	10.28	
	40	0.621	17.42	
	80	0.555	26.17	
	160	0.423	43.75	
	320	0.284	62.23	



# Conclusion

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Ellagic acid is a natural polyphenol component that is present in Shilajit and has the most associations with cancer related diseases. Docking studies done on ellagic acid, a component of Shilajit shows that it binds with the cancer targets PTGS2 and EGFR in a stable conformation. The crude extract of Shilajit shows that when tested on cancer cell lines, it has a % inhibition value of 62.23. The present study indicates that Shilajit might be of value as sources or leads for novel anti-cancer drugs.

# Scope

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Network pharmacology aims to understand the network interactions between a living organism and drugs that affect normal or abnormal biochemical function. It tries to exploit the pharmacological mechanism of drug action in the biological network, and helps to find drug targets and enhance the drug's efficacy. Therefore, this approach can initiate new directions and lead to a probable revolution in the modernization of network drugs, and also provide new insights into the current drug discovery field.

# Plan of Work

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Phase	Period	Work to be executed
1	Literature Mining	Jan 29 <sup>th</sup> – Feb 15 <sup>th</sup>
2	Finding bio-actives & targets	Feb 15 <sup>th</sup> – Feb 28 <sup>th</sup>
3	Network Construction	March 1 <sup>st</sup> – April 1 <sup>st</sup>
4	Validation	April 1 <sup>st</sup> – April 20 <sup>th</sup>

# Selected References

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- [1]E. Wilson et al., "Review on shilajit used in traditional Indian medicine", *Journal of Ethnopharmacology*, vol. 136, no. 1, pp. 1-9, 2011. Available: 10.1016/j.jep.2011.04.033.
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- [3]U. Chandran, N. Mehendale, G. Tillu and B. Patwardhan, "Network Pharmacology of Ayurveda Formulation Triphala with Special Reference to Anti-Cancer Property", *Combinatorial Chemistry & High Throughput Screening*, vol. 18, no. 9, pp. 846-854, 2015. Available: 10.2174/1386207318666151019093606.
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- [6]J. Keller, T. Housh, E. Hill, C. Smith, R. Schmidt and G. Johnson, "The effects of Shilajit supplementation on fatigue-induced decreases in muscular strength and serum hydroxyproline levels", *Journal of the International Society of Sports Nutrition*, vol. 16, no. 1, 2019. Available: 10.1186/s12970-019-0270-2.
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