Cytoscape A Tool to Visualize Protein-Protein interactions using Eugenol, a Phytochemical of *Ocimum sanctum* L

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ABSTRACT

Eugenol (4-hydroxy-3-methoxy-allylbenzene) is an important bioactive compound found in various medicinal plants and has been extensively investigated for its pharmacological properties. Apart from its anaesthetic and analgesic properties, this phenolic compound has antimicrobial, anti-inflammatory, anticancer and antioxidant properties. One of the promising candidates with versatile applications in designing new drugs will be Eugenol for its pharmacological effects. While the metabolism and pharmacokinetics of Eugenol have been studied in humans, still there is lack of data available on its precise mode of action and its impact on system level protein networks. To identify the action of Eugenol on protein interaction network, an interactome was constructed using STRING and Cytoscape tools, based on 98 key proteins extracted from PubMed literatures. Swiss Target Prediction was used to predict the possible macromolecular targets in humans. It was predicted that the target classes included 20% enzymes, secreted protein, and Family A G- protein coupled receptors, 13.3% of Ligand-gated ion channel and 6.7% of kinase, eraser, lyase and oxidoreductases. Further, using the Cytoscape 3.9.1 version *in-silico* study, it could be observed that 92 nodes in total were connected with 61 edges with a clustering coefficient of 0.369. Using a phytochemical Eugenol, the possible network analysis has been predicted. Evaluating the network among the other phytochemicals of *Ocimum sanctum* could help us to investigate the role played by these key phytochemicals at gene and molecular level and can help us explore their interference in disease patterns in humans.

Keywords: *Cytoscape 3.9.1 version, Edges, Nodes, String database*

1. INTRODUCTION

From ancient times medicinal plants have been used to fight different types of diseases (Petrovska 2012). To manage diseases, bioactive compounds derived naturally has wide potential in medicinal and also as therapeutic agents. To maintain a balance within the cell, certain important phytochemicals present in the medicinal plants act as multiple protein targets (Ding et al. 2009). Eugenol is an example of a phytochemical present in the leaves of *Ocimum sanctum*. The molecule's extensive *in vitro* and *in vivo* reports as a therapeutic agent, and the lack of experimental data on its interactions with proteins, make it a compelling study target (Huang et al. 2018).

Modern molecular biology requires biologists to focus on biomolecular components, such as Eugenol, and their interaction with other proteins via a complex web system in a living cell (Hartwell et al. 1999). Protein-protein interactions (PPIs) are the most common type of macromolecular interaction in a living system. PPIs are dynamic in nature and adjust according to the varying stimuli and environmental conditions. Eugenol has been reported to interact with various proteins to alter their function. For instance, Eugenol increases Vitamin D3 upregulated protein 1 (VDUPI) and IκBα levels in the NF-κB pathway, displaying anti-asthmatic effect (Pan and Dong, 2015).

To study these complex proteome networks, graph theory and advanced system biology approaches are employed by researchers. System biology, as an interdisciplinary field, can bridge the gap between *in vitro* and *in vivo* models through the data procured from *omics* and reveal the function of biological micro and macromolecules present in any biological model (Koutsogiannouli et al. 2013). With the currently available *in-silico* tools, screening and analyzing the network via pharmacological aspects of natural plant products like phytochemicals can be performed to understand the multi-targeted action of plant metabolites, to create a protein network system (Chandran et al. 2017).

Cytoscape version 3.9.1 was developed to understand the interactions among proteins at molecular level and create a profile of networks that can be used to visualize all possible molecular interactions within the networks (Shannon et al. 2003, Smoot et al. 2010). Developing networks reduces the complexity in data, is more efficient than table interpretations, and data integration can be done at optimal level with attractive visualization features. Biological Network Taxonomy can be categorized into metabolic, signalling and regulatory pathways and diseases.

Constructing the PINs (Protein Interaction Networks) of Eugenol involved screening the phytochemical compound from PubChem database and saving the structure as its SMILES notation. A network can be created to predict the interactions between phytochemicals and proteins, protein targets, and enriched pathways using the Swiss Target Prediction tool. This allows us to predict the protein-protein interactions with which a downstream analysis can be performed. Using String database, the data related to phytochemical interaction with proteins present in humans can be exported to Cytoscape tool to analyze, visualize, and interpret the outcome. These networks can be sorted based on organelles, tissues, text mining of the data, clustering coefficients, neighbourhood's, in betweenness, number of nodes and edges, etc. With the available output, the major proteins involved in interactions can be shortlisted about eugenol.

2. MATERIALS AND METHODS

The workflow includes the use of following tools,

- Retrieve the phytochemical structure and SMILES notation from PubChem database. https://pubchem.ncbi.nlm.nih.gov/
- Screening the probable macromolecular targets of small molecules assumed as bioactive using Swiss Target Prediction version 11.5. It predicts both the two- and three-dimensional (2D and 3D) similarity among the 3,70,000 libraries of known actives of nearly 3,000 different proteins available on three different animal species. http://swisstargetprediction.ch/
- Screening for the protein-protein interactions using string database. https://string-db.org/
- Building networks using Cytoscape network tool 3.9.1 version.
- Analyse and interpret the data

2.1. Protein-Protein Interaction Network Construction and Visualization

The SMILES notation for Eugenol was submitted to the online Swiss Target Prediction tool, and a set of

target proteins were obtained for *Homo sapiens*. The retrieved data was saved as an .csv file. The excel sheet obtained contained the set of proteins with target, common name, ChEMBL ID code, Uniport ID, Target class, known active 2D and 3D from Swiss Target Prediction (Fig. 1).

The common names of all possible protein targets were submitted into the string database (version 11.5), a tool used to predict all possible protein-protein interactions. Selecting the multiple proteins and feeding their common names into string database gave a visual output network with all possible interactions (Fig. 3). The obtained results were retrieved and stored in an .csv format. From the obtained Functional Enrichment data, KEGG pathway output was selected and exported to Cytoscape tool for further analysis.

2.2. Protein Interaction Network Analysis

The interaction network in Cytoscape was analyzed using its inbuilt Network Analyzer plugin, which gave the primary topological framework of Eugenol-rewired PINs, like clustering coefficient, network density, network heterogeneity, network centralization, and connected components. MCODE plug-in of Cytoscape was applied for modulation of the network (finding highly interconnected regions or clusters in the network). Clusters in PINs are often metabolic pathways and protein complexes (Bader and Hogue 2003). Cytoscape scored each module based on density and size, incase if the output is with higher score probably it indicates a possible tighter module.

3. RESULTS AND DISCUSSION

3.1. Retrieving Target Proteins

To retrieve the SMILES notation of Eugenol [COC1=C(C=CC(=C1)CC=C)] interaction with proteins in *Homo sapiens* PubChem online portal was exercised. 101 possible protein targets were retrieved by submitting the SMILES notation in the online Swiss Target Prediction Tool (TABLE 1). Out of these targets 20% belonged to Family A G-protein coupled receptor, enzymes, and secreted proteins each, while 6.7 % were Erasers, oxidoreductases, lyases, and kinases. Ligand-gated ion channels covered the remaining 13.3% of targets (Fig. 2).

3.2. Network Construction in STRING

STRING database was used to generate the PINs rewired by Eugenol by inputting the common names of targets retrieved from Swiss Target Prediction Tool. The 98 nodes or proteins in the network output (Fig. 4) covered 81 different GO molecular functions, 124 KEGG pathways, 414 interactions (physical or functional). The network statistics indicated an average node degree, local clustering coefficient of 7.67 and 0.583 respectively with a PPI enrichment p-value $<1.0e^{-16}$ (Table 2 & 3).

3.3. Topology and Network Modulation in Cytoscape

The Protein Interaction Network of an intricate cellular system can be studied through its topology, which can reveal the connections and interactions of biological macromolecules in various pathways that is metabolic and cellular in nature (Ba et al. 2015). This study was focused on, understanding the gene/protein connection pathways that was altered by Eugenol, and Cytoscape's Network Analyser plug-in was applied for topological analysis (Fig. 5) (Table 4).

The shortest path length for a Protein Interaction Network shows the number of edges through the shortest paths between two nodes. On the other hand, closeness centrality is the inverse of the average shortest path. These parameters outline the ability to transport information and the comprehensive navigability of the interactome (Ba *et al.* 2015, Delprato 2012). The characteristic pathlength for Eugenol-rewired PIN was 2.809 (Fig. 6).

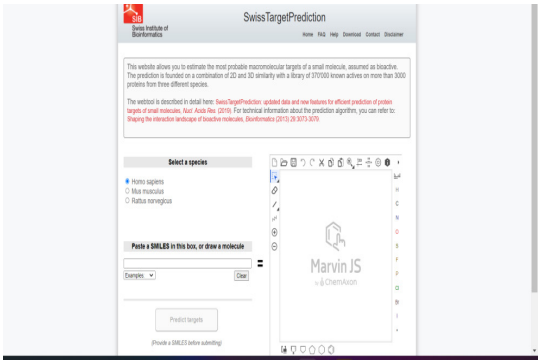
Clustering coefficient tells us how close the nodes are to their neighbours and the hierarchical modularity of the PIN. It is used to identify the potential functional modules and unwrap the complexity in molecular and signalling pathways in the network (Bader and Hogue 2003, Ba et al. 2015, Barabási and Oltvai 2004). For the current PIN generated with Eugenol, 0.369 was found to be the clustering coefficient distribution (Fig. 7). Plug-in MCODE was used to work out 5 modules from the KEGG PIN rewired by Eugenol (Fig. 8).

4. CONCLUSION

With a simple workflow, Cytoscape tool was used to predict and understand the number of protein- protein interactions possible within the selected phytochemical Eugenol. This visual network data output was in terms of subcellular locations and pharmacokinetic data. From the above study it was observed that, the KEGG enriched PIN has 92 nodes with 61 edges

and a clustering coefficient of 0.369 (TABLE 4). Eugenol, has been recognized as a safe food additive under food substance category by Federal Food, Drug and Cosmetic administration as it could work synergistically with various antimicrobial compounds present in foods. Further, researchers are designing and developing eugenol structure-based derivatives predicting to have different structural and biological properties. While the current study only focused on one phytochemical (Eugenol), protein network analysis of other phytochemicals can help us understand the role played by them at gene and molecular level. They can also provide an insight into their interference in disease patterns in human.

5. TABLES AND FIGURES



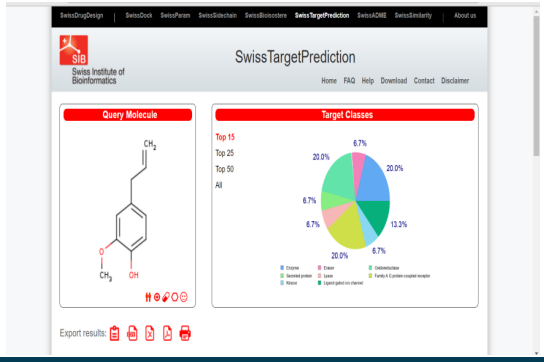


Fig.1: Swiss Target Prediction Tool Input and Output

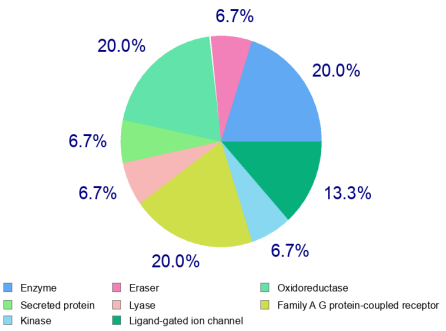
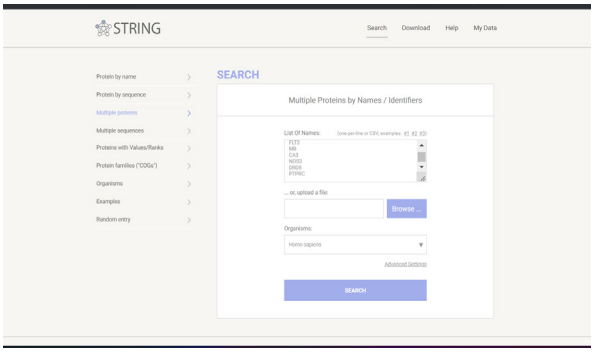


Fig.2: Target Classes of Eugenol



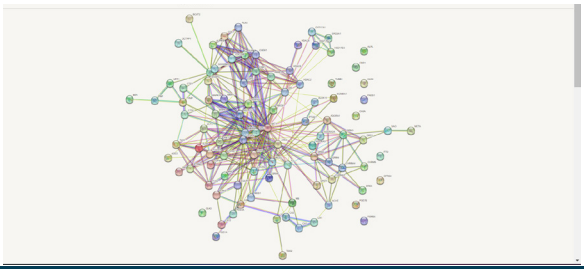


Fig.3: STRING Database Input and Output. Potential protein targets of Eugenol were retrieved from Swiss Target Prediction Tool and were entered in the STRING prompt, specific for Humans (left). The output displayed a complex interactome among the proteins retrieved (right).

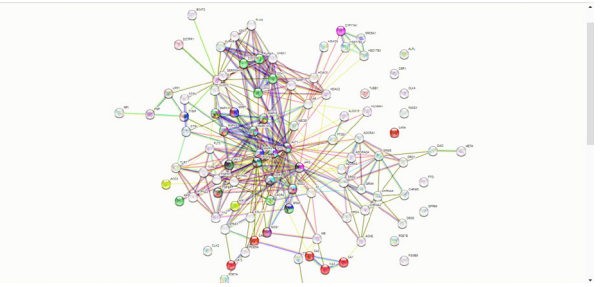


Fig.4: Protein Interaction Network (PIN in STRING). The PIN generated in STRING displays all the possible interactions among the potential protein targets for Eugenol

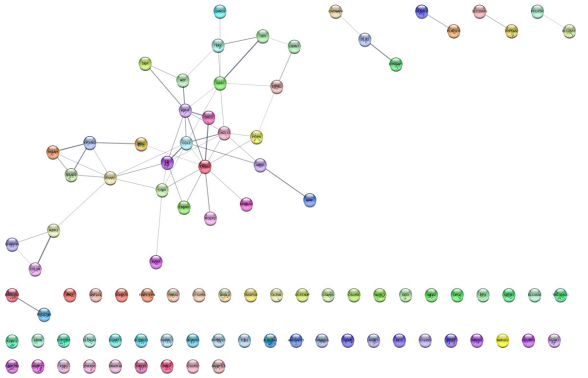


Fig.5: KEGG Enriched PIN in Cytoscape. The above PIN displays interactions between potential Eugenol targets that are found in various KEGG pathways

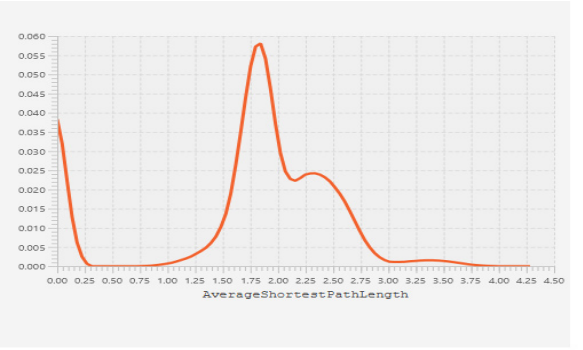


Fig. 6: Characteristic Pathlength is 2.809. Histogram generated by Cytoscape Network Analyzer. The y-axis represents the frequency of the average shortest path lengths found in the interaction network.

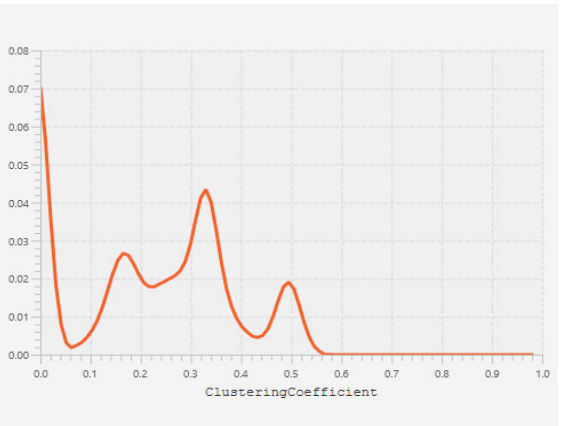


Fig.7: Clustering coefficient distribution is 0.369. Histogram generated by Cytoscape Network Analyzer. The y-axis represents the average clustering coefficient.

Fig.8: Cluster modules obtained through MCODE. The five clusters found may represent functionally homogenous sub-networks of proteins within the large PIN, based on their densely interconnected nodes.

Table 1: Possible protein targets for Eugenol obtained from Swiss Target Prediction tool

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability* Probability for the query molecule - assumed as bioactive - to have this protein as target.
Fatty acid desaturase 1	FADS1	O60427	CHEMBL5840	Enzyme	0.133391
Histone deacetylase 6	HDAC6	Q9UBN7	CHEMBL1865	Eraser	0.133391
Egl nine homolog 1	EGLN1	Q9GZT9	CHEMBL5697	Oxidoreductase	0.125076
Vascular endothelial growth factor A	VEGFA	P15692	CHEMBL1783	Secreted protein	0.125076
Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	0.125076
G-protein coupled receptor 84	GPR84	Q9NQS5	CHEMBL3714079	Family A G protein-coupled receptor	0.125076
Cyclooxygenase-1	PTGS1	P23219	CHEMBL221	Oxidoreductase	0.125076
D-amino-acid oxidase	DAO	P14920	CHEMBL5485	Enzyme	0.125076
Poly [ADP-ribose] polymerase-1	PARP1	P09874	CHEMBL3105	Enzyme	0.125076
Tyrosine-protein kinase SRC	SRC	P12931	CHEMBL267	Kinase	0.125076
Adenosine A1 receptor	ADORA1	P30542	CHEMBL226	Family A G protein-coupled receptor	0.125076
Adenosine A2a receptor	ADORA2A	P29274	CHEMBL251	Family A G protein-coupled receptor	0.125076
Steroid 5-alpha-reductase 1	SRD5A1	P18405	CHEMBL1787	Oxidoreductase	0.125076
Neuronal acetylcholine receptor subunit alpha-3	CHRNA3	P32297	CHEMBL3068	Ligand-gated ion channel	0.125076
Neuronal acetylcholine receptor protein alpha-4 subunit (by homology)	CHRNA4	P43681	CHEMBL1882	Ligand-gated ion channel	0.125076
Interleukin-8 receptor B	CXCR2	P25025	CHEMBL2434	Family A G protein-coupled receptor	0.125076
dCTP pyrophosphatase 1	DCTPP1	Q9H773	CHEMBL3769292	Enzyme	0.125076
Alkaline phosphatase, tissue-nonspecific isozyme	ALPL	P05186	CHEMBL5979	Enzyme	0.125076
Methionine aminopeptidase 2	METAP2	P50579	CHEMBL3922	Protease	0.116739
Carbonyl reductase [NADPH] 1	CBR1	P16152	CHEMBL5586	Enzyme	0.116739
Calcium-activated potassium channel subunit alpha-1	KCNMA1	Q12791	CHEMBL4304	Voltage-gated ion channel	0.116739
Arachidonate 15-lipoxygenase	ALOX15	P16050	CHEMBL2903	Enzyme	0.116739

Neuronal acetylcholine receptor; alpha3/beta4	CHRNA3 CHRNB4	P32297 P30926	CHEMBL1907594	Ligand-gated ion channel	0.116739
Vascular endothelial growth factor receptor 2	KDR	P35968	CHEMBL279	Kinase	0.116739
Histone deacetylase 8	HDAC8	Q9BY41	CHEMBL3192	Eraser	0.116739
Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor	0.116739
Proteasome Macropain subunit MB1	PSMB5	P28074	CHEMBL4662	Protease	0.116739
Tryptophan 2,3-dioxygenase (by homology)	TDO2	P48775	CHEMBL2140	Enzyme	0.116739
Neuronal acetylcholine receptor; alpha4/beta2	CHRNA4 CHRNB2	P43681 P17787	CHEMBL1907589	Ligand-gated ion channel	0.116739
Estradiol 17-beta-dehydrogenase 3	HSD17B3	P37058	CHEMBL4234	Enzyme	0.116739
Cytochrome P450 17A1 (by homology)	CYP17A1	P05093	CHEMBL3522	Cytochrome P450	0.116739
Serine/threonine-protein kinase Aurora-A	AURKA	O14965	CHEMBL4722	Kinase	0.116739
Metabotropic glutamate receptor 5	GRM5	P41594	CHEMBL3227	Family C G protein-coupled receptor	0
Transthyretin	TTR	P02766	CHEMBL3194	Secreted protein	0
Estradiol 17-beta-dehydrogenase 2	HSD17B2	P37059	CHEMBL2789	Enzyme	0
Dopamine D2 receptor	DRD2	P14416	CHEMBL217	Family A G protein-coupled receptor	0
Hematopoietic cell protein-tyrosine phosphatase 70Z-PEP	PTPN22	Q9Y2R2	CHEMBL2889	Phosphatase	0
Toll-like receptor (TLR7/TLR9)	TLR9	Q9NR96	CHEMBL5804	Toll-like and Il-1 receptors	0
Tubulin beta-1 chain	TUBB1	Q9H4B7	CHEMBL1915	Structural protein	0
Tyrosine-protein kinase JAK1	JAK1	P23458	CHEMBL2835	Kinase	0
Tyrosine-protein kinase JAK2	JAK2	O60674	CHEMBL2971	Kinase	0
Tyrosine-protein kinase TYK2	TYK2	P29597	CHEMBL3553	Kinase	0
Serine/threonine-protein kinase B-raf	BRAF	P15056	CHEMBL5145	Kinase	0
Thymidylate synthase	TYMS	P04818	CHEMBL1952	Transferase	0
Uridine phosphorylase 1 (by homology)	UPP1	Q16831	CHEMBL4811	Enzyme	0
Mannose-6-phosphate isomerase	MPI	P34949	CHEMBL2758	Isomerase	0
Dual specificity protein phosphatase 3	DUSP3	P51452	CHEMBL2635	Phosphatase	0
Phosphodiesterase 7A	PDE7A	Q13946	CHEMBL3012	Phosphodiesterase	0
Fibroblast growth factor receptor 1	FGFR1	P11362	CHEMBL3650	Kinase	0
Carbonic anhydrase VA	CA5A	P35218	CHEMBL4789	Lyase	0
Ribosomal protein S6 kinase alpha 3	RPS6KA3	P51812	CHEMBL2345	Kinase	0

Matrix metalloproteinase 9	MMP9	P14780	CHEMBL321	Protease	0
Nitric-oxide synthase, brain	NOS1	P29475	CHEMBL3568	Enzyme	0
Serine/threonine-protein kinase Chk1	CHEK1	O14757	CHEMBL4630	Kinase	0
Muscarinic acetylcholine receptor M5	CHRM5	P08912	CHEMBL2035	Family A G protein-coupled receptor	0
Cyclin-dependent kinase 2	CDK2	P24941	CHEMBL301	Kinase	0
Thymidine phosphorylase	TYMP	P19971	CHEMBL3106	Enzyme	0
Toll-like receptor (TLR7/ TLR9)	TLR7	Q9NYK1	CHEMBL5936	Toll-like and Il-1 receptors	0
Endothelial PAS domain-containing protein 1	EPAS1	Q99814	CHEMBL1744522	Unclassified protein	0
Matrix metalloproteinase 1	MMP1	P03956	CHEMBL332	Protease	0
Phosphodiesterase 5A	PDE5A	O76074	CHEMBL1827	Phosphodiesterase	0
c-Jun N-terminal kinase 1	MAPK8	P45983	CHEMBL2276	Kinase	0
c-Jun N-terminal kinase 3	MAPK10	P53779	CHEMBL2637	Kinase	0
c-Jun N-terminal kinase 2	MAPK9	P45984	CHEMBL4179	Kinase	0
Thrombin	F2	P00734	CHEMBL204	Protease	0
Serine/threonine-protein kinase AKT	AKT1	P31749	CHEMBL4282	Kinase	0
Dual specificity phosphatase Cdc25B	CDC25B	P30305	CHEMBL4804	Phosphatase	0
Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	0
Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase	0
Carbonic anhydrase IX	CA9	Q16790	CHEMBL3594	Lyase	0
Serine/threonine-protein kinase PLK4	PLK4	O00444	CHEMBL3788	Kinase	0
Dual specificity protein kinase CLK4	CLK4	Q9HAZ1	CHEMBL4203	Kinase	0
Dual specificity protein kinase CLK2	CLK2	P49760	CHEMBL4225	Kinase	0
Amine oxidase, copper containing	AOC3	Q16853	CHEMBL3437	Enzyme	0
P-glycoprotein 1	ABCB1	P08183	CHEMBL4302	Primary active transporter	0
Histone deacetylase 2	HDAC2	Q92769	CHEMBL1937	Eraser	0
TGF-beta receptor type I	TGFBR1	P36897	CHEMBL4439	Kinase	0
MAP kinase p38 alpha	MAPK14	Q16539	CHEMBL260	Kinase	0
Metabotropic glutamate receptor 4	GRM4	Q14833	CHEMBL2736	Family C G protein-coupled receptor	0
Transmembrane domain-containing protein TMIGD3	TMIGD3	P0DMS9	CHEMBL3712907	Unclassified protein	0
Acetylcholinesterase	ACHE	P22303	CHEMBL220	Hydrolase	0
Macrophage migration inhibitory factor	MIF	P14174	CHEMBL2085	Enzyme	0

Dopamine D1 receptor	DRD1	P21728	CHEMBL2056	Family A G protein-coupled receptor	0
Branched-chain-amino-acid aminotransferase, mitochondrial	BCAT2	O15382	CHEMBL3616354	Transferase	0
Serine/threonine-protein kinase Aurora-B	AURKB	Q96GD4	CHEMBL2185	Kinase	0
Cyclin-dependent kinase 1	CDK1	P06493	CHEMBL308	Kinase	0
Collagen-binding protein 1	SERPINH1	P50454	CHEMBL5286	Other cytosolic protein	0
Cathepsin (V and K)	CTSV	O60911	CHEMBL3272	Protease	0
Cathepsin L	CTSL	P07711	CHEMBL3837	Protease	0
CDC7/DBF4 (Cell division cycle 7-related protein kinase/ Activator of S phase kinase)	CDC7	O00311	CHEMBL5443	Kinase	0
Phosphodiesterase 7B	PDE7B	Q9NP56	CHEMBL4716	Phosphodiesterase	0
Alpha-ketoglutarate-dependent dioxygenase FTO	FTO	Q9C0B1	CHEMBL2331065	Oxidoreductase	0
Purine nucleoside phosphorylase	PNP	P00491	CHEMBL4338	Enzyme	0
Dopamine D4 receptor	DRD4	P21917	CHEMBL219	Family A G protein-coupled receptor	0
Tyrosine-protein kinase receptor FLT3	FLT3	P36888	CHEMBL1974	Kinase	0
Myoglobin	MB	P02144	CHEMBL2406892	Unclassified protein	0
Carbonic anhydrase III	CA3	P07451	CHEMBL2885	Lyase	0
Nitric-oxide synthase, endothelial	NOS3	P29474	CHEMBL4803	Enzyme	0
Dopamine D5 receptor	DRD5	P21918	CHEMBL1850	Family A G protein-coupled receptor	0
Leukocyte common antigen	PTPRC	P08575	CHEMBL3243	Enzyme	0

Table 2: Network statistics of PIN from STRING

Network Statistics	Results
Number of Nodes	98
Number of Edges	365
Average Node Degree	7.24
Average local clustering coefficient	0.559
Expected Number of Edges	161
PPI Enrichment p-value	$<1.0e^{-16}$

Table 3: KEGG functional enrichment output from STRING

Pathway	Description	Count in network	Strength	False discovery in data
hsa00910	Nitrogen metabolism	6 of 17	1.86	4.58E-08
hsa05219	Bladder cancer	6 of 41	1.47	1.87E-06
hsa04914	Progesterone-mediated oocyte maturation	11 of 94	1.38	8.03E-10
hsa00360	Phenylalanine metabolism	2 of 17	1.38	0.0127
hsa04917	Prolactin signaling pathway	8 of 69	1.37	1.29E-07
hsa05212	Pancreatic cancer	8 of 73	1.35	1.76E-07
hsa04370	VEGF signaling pathway	6 of 57	1.33	8.11E-06
hsa04215	Apoptosis - multiple species	3 of 30	1.31	0.0024
hsa00220	Arginine biosynthesis	2 of 21	1.29	0.0169
hsa04926	Relaxin signaling pathway	12 of 128	1.28	8.03E-10
hsa04933	AGE-RAGE signaling pathway in diabetic complications	9 of 98	1.27	9.71E-08
hsa01521	EGFR tyrosine kinase inhibitor resistance	7 of 78	1.26	2.93E-06
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	6 of 67	1.26	1.54E-05
hsa00240	Pyrimidine metabolism	5 of 56	1.26	8.59E-05
hsa05418	Fluid shear stress and atherosclerosis	11 of 130	1.24	6.13E-09
hsa01522	Endocrine resistance	8 of 95	1.23	9.37E-07
hsa05161	Hepatitis B	13 of 159	1.22	8.03E-10
hsa04659	Th17 cell differentiation	8 of 101	1.21	1.20E-06
hsa04658	Th1 and Th2 cell differentiation	7 of 87	1.21	5.16E-06
hsa00790	Folate biosynthesis	2 of 25	1.21	0.0222
hsa05145	Toxoplasmosis	8 of 105	1.19	1.51E-06
hsa05211	Renal cell carcinoma	5 of 66	1.19	0.00015
hsa04664	Fc epsilon RI signaling pathway	5 of 66	1.19	0.00015
hsa05210	Colorectal cancer	6 of 82	1.17	4.01E-05
hsa04012	ErbB signaling pathway	6 of 83	1.17	4.18E-05
hsa04920	Adipocytokine signaling pathway	5 of 69	1.17	0.00018
hsa04728	Dopaminergic synapse	9 of 128	1.16	6.06E-07
hsa04068	FoxO signaling pathway	9 of 127	1.16	6.06E-07
hsa05142	Chagas disease	7 of 99	1.16	1.01E-05
hsa04620	Toll-like receptor signaling pathway	7 of 101	1.15	1.07E-05
hsa04540	Gap junction	6 of 87	1.15	5.13E-05
hsa00140	Steroid hormone biosynthesis	4 of 59	1.14	0.0012
hsa04380	Osteoclast differentiation	8 of 122	1.13	3.35E-06
hsa05162	Measles	9 of 138	1.12	9.53E-07
hsa04657	IL-17 signaling pathway	6 of 92	1.12	6.63E-05

hsa04930	Type II diabetes mellitus	3 of 46	1.12	0.0062
hsa05215	Prostate cancer	6 of 96	1.11	7.97E-05
hsa04137	Mitophagy - animal	4 of 63	1.11	0.0015
hsa04722	Neurotrophin signaling pathway	7 of 114	1.1	2.10E-05
hsa04071	Sphingolipid signaling pathway	7 of 116	1.09	2.28E-05
hsa04660	T cell receptor signaling pathway	6 of 101	1.08	9.64E-05
hsa04625	C-type lectin receptor signaling pathway	6 of 102	1.08	9.96E-05
hsa04622	RIG-I-like receptor signaling pathway	4 of 70	1.07	0.0021
hsa00350	Tyrosine metabolism	2 of 35	1.07	0.0394
hsa05135	Yersinia infection	7 of 125	1.06	3.55E-05
hsa04931	Insulin resistance	6 of 107	1.06	0.00013
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	5 of 88	1.06	0.00046
hsa04912	GnRH signaling pathway	5 of 89	1.06	0.00047
hsa04725	Cholinergic synapse	6 of 110	1.05	0.00014
hsa04923	Regulation of lipolysis in adipocytes	3 of 54	1.05	0.0093
hsa05167	Kaposi sarcoma-associated herpesvirus infection	10 of 187	1.04	9.53E-07
hsa05152	Tuberculosis	9 of 168	1.04	3.06E-06
hsa04217	Necroptosis	8 of 149	1.04	1.07E-05
hsa04668	TNF signaling pathway	6 of 112	1.04	0.00015
hsa04750	Inflammatory mediator regulation of TRP channels	5 of 94	1.04	0.00059
hsa05220	Chronic myeloid leukemia	4 of 75	1.04	0.0025
hsa05133	Pertussis	4 of 74	1.04	0.0025
hsa04210	Apoptosis	7 of 132	1.03	4.50E-05
hsa04935	Growth hormone synthesis, secretion and action	6 of 118	1.02	0.00019
hsa04114	Oocyte meiosis	6 of 120	1.01	0.0002
hsa04110	Cell cycle	6 of 120	1.01	0.0002
hsa04010	MAPK signaling pathway	14 of 288	1	1.23E-08
hsa05034	Alcoholism	7 of 144	1	7.04E-05
hsa04611	Platelet activation	6 of 122	1	0.00021
hsa00590	Arachidonic acid metabolism	3 of 61	1	0.0127
hsa04024	cAMP signaling pathway	10 of 208	0.99	1.87E-06
hsa05169	Epstein-Barr virus infection	9 of 193	0.98	8.11E-06
hsa04720	Long-term potentiation	3 of 64	0.98	0.0139
hsa05205	Proteoglycans in cancer	9 of 196	0.97	8.68E-06
hsa05221	Acute myeloid leukemia	3 of 66	0.97	0.0147
hsa04015	Rap1 signaling pathway	9 of 202	0.96	1.03E-05
hsa05032	Morphine addiction	4 of 89	0.96	0.0042
hsa04520	Adherens junction	3 of 67	0.96	0.0152
hsa05203	Viral carcinogenesis	8 of 182	0.95	3.82E-05
hsa05230	Central carbon metabolism in cancer	3 of 69	0.95	0.0163

hsa05140	Leishmaniasis	3 of 70	0.94	0.0167
hsa05231	Choline metabolism in cancer	4 of 96	0.93	0.0053
hsa05218	Melanoma	3 of 72	0.93	0.0176
hsa04115	p53 signaling pathway	3 of 72	0.93	0.0176
hsa04510	Focal adhesion	8 of 198	0.92	6.11E-05
hsa04218	Cellular senescence	6 of 150	0.91	0.00056
hsa05166	Human T-cell leukemia virus 1 infection	8 of 211	0.89	8.59E-05
hsa04140	Autophagy - animal	5 of 130	0.89	0.0023
hsa04066	HIF-1 signaling pathway	4 of 106	0.89	0.0073
hsa05130	Pathogenic Escherichia coli infection	7 of 187	0.88	0.00025
hsa04910	Insulin signaling pathway	5 of 133	0.88	0.0025
hsa05017	Spinocerebellar ataxia	5 of 135	0.88	0.0026
hsa05200	Pathways in cancer	19 of 517	0.87	9.25E-10
hsa04080	Neuroactive ligand-receptor interaction	12 of 330	0.87	1.87E-06
hsa04020	Calcium signaling pathway	7 of 193	0.87	0.0003
hsa04014	Ras signaling pathway	8 of 226	0.86	0.00013
hsa04550	Signaling pathways regulating pluripotency of stem cells	5 of 140	0.86	0.003
hsa05323	Rheumatoid arthritis	3 of 85	0.86	0.0259
hsa04621	NOD-like receptor signaling pathway	6 of 174	0.85	0.0012
hsa05226	Gastric cancer	5 of 144	0.85	0.0032
hsa04723	Retrograde endocannabinoid signaling	5 of 145	0.85	0.0033
hsa05170	Human immunodeficiency virus 1 infection	7 of 204	0.84	0.0004
hsa04072	Phospholipase D signaling pathway	5 of 147	0.84	0.0034
hsa05012	Parkinson disease	8 of 240	0.83	0.00017
hsa05132	Salmonella infection	7 of 209	0.83	0.00046
hsa05160	Hepatitis C	5 of 156	0.82	0.0043
hsa00230	Purine metabolism	4 of 127	0.81	0.0128
hsa05206	MicroRNAs in cancer	5 of 160	0.8	0.0048
hsa04022	cGMP-PKG signaling pathway	5 of 162	0.8	0.005
hsa05164	Influenza A	5 of 165	0.79	0.0053
hsa04371	Apelin signaling pathway	4 of 131	0.79	0.0139
hsa04915	Estrogen signaling pathway	4 of 133	0.79	0.0145
hsa05010	Alzheimer disease	10 of 355	0.76	8.59E-05
hsa05163	Human cytomegalovirus infection	6 of 218	0.75	0.0032
hsa05131	Shigellosis	6 of 218	0.75	0.0032
hsa04726	Serotonergic synapse	3 of 108	0.75	0.0475
hsa04062	Chemokine signaling pathway	5 of 186	0.74	0.0084
hsa04932	Non-alcoholic fatty liver disease	4 of 148	0.74	0.0192
hsa04151	PI3K-Akt signaling pathway	9 of 350	0.72	0.00034

hsa04530	Tight junction	4 of 156	0.72	0.0226
hsa04630	JAK-STAT signaling pathway	4 of 160	0.71	0.0244
hsa04810	Regulation of actin cytoskeleton	5 of 209	0.69	0.013
hsa05016	Huntington disease	7 of 298	0.68	0.003
hsa05020	Prion disease	6 of 265	0.66	0.0074
hsa01100	Metabolic pathways	28 of 1447	0.6	1.23E-08
hsa05165	Human papillomavirus infection	6 of 325	0.96	0.0042
hsa04520	Adherens junction	3 of 67	0.96	0.0152
hsa05203	Viral carcinogenesis	8 of 182	0.95	3.82E-05
hsa05230	Central carbon metabolism in cancer	3 of 69	0.95	0.0163
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hsa05226	Gastric cancer	5 of 144	0.85	0.0032
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hsa05160	Hepatitis C	5 of 156	0.82	0.0043
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hsa04371	Apelin signaling pathway	4 of 131	0.79	0.0139
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hsa05010	Alzheimer disease	10 of 355	0.76	8.59E-05
hsa05163	Human cytomegalovirus infection	6 of 218	0.75	0.0032
hsa05131	Shigellosis	6 of 218	0.75	0.0032
hsa04726	Serotonergic synapse	3 of 108	0.75	0.0475
hsa04062	Chemokine signaling pathway	5 of 186	0.74	0.0084
hsa04932	Non-alcoholic fatty liver disease	4 of 148	0.74	0.0192
hsa04151	PI3K-Akt signaling pathway	9 of 350	0.72	0.00034
hsa04530	Tight junction	4 of 156	0.72	0.0226
hsa04630	JAK-STAT signaling pathway	4 of 160	0.71	0.0244
hsa04810	Regulation of actin cytoskeleton	5 of 209	0.69	0.013
hsa05016	Huntington disease	7 of 298	0.68	0.003
hsa05020	Prion disease	6 of 265	0.66	0.0074
hsa01100	Metabolic pathways	28 of 1447	0.6	1.23E-08
hsa05165	Human papillomavirus infection	6 of 325	0.58	0.017

Table 4: Summary statistics on Eugenol PIN (KEGG enriched) in Cytoscape

Number of Nodes	92
Number of Edges	61
Average number of neighbours	3.667
Network Diameter	6
Network Radius	3
Characteristic path length	2.809
Clustering coefficient	0.369
Network Density	0.126
Network heterogeneity	0.680
Network Centralization	0.234
Connected components	57
Analysis time	0.0032

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DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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