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Research Article

NETWORK PHARMACOLOGY AND MOLECULAR DOCKING-BASED PREDICTIONS OF PHARMACOLOGICAL EFFECTS OF FERULIC ACID

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ABSTRACT

Objectives: The main objective of this study is to reveal new possible pharmacological effects of ferulic acid. This is achieved by network pharmacology by discovering potential target genes for ferulic acid, along with constructing a PPI network for those targets and performing gene enrichment analysis to understand possible diseases or disorders being affected due to the target genes. The study involves the molecular docking of target genes with ferulic acid to understand the interactions between them.

Methods: ADMETlab 2.0 was used for the pharmacokinetics study of ferulic acid. Using SwissTargetPrediction and STITCH database 79 target genes were retrieved which were used to construct a PPI network using the STRING database and for gene enrichment analysis using the ShinyGo tool. Analyzing the clusters generated by k-means clustering in the STRING database, three target gene proteins were further used to perform molecular docking with ferulic acid using PyRx software, and 2D and 3D visualization was done using Biovia Discovery Studio Visualizer.

Results: The ADMET analysis ferulic acid showed drug-likeliness. SwissTargetPrediction and STITCH database revealed 79 potential target genes. Three proteins (RELA, ALOX15, and STAT3) were selected from the PPI network analysis using the STRING database for molecular docking and visualization. ALOX15 showed the least binding energy among all three target proteins. Gene enrichment analysis suggests the target proteins are involved in cancer, neurological disorders, psychiatric disorders, Alzheimer's disease, etc.

Conclusion: The findings of this research suggest that ferulic acid may have a wide range of pharmacological effects and gives a new perspective on its application in the field of drug discovery.

Keywords: Network pharmacology, Molecular docking, Ferulic acid, ADMET, Cancer, Neurological disorders

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INTRODUCTION

Plants produce a variety of secondary metabolites that are crucial for ecology, environmental adaptability, and plant defense systems but are not necessary for normal development and reproduction [1]. The biological effects of these substances include antibacterial activity, antioxidant effects, anticancer effects, modulation of detoxification enzymes, immune system activation, a reduction in platelet aggregation, and modification of hormone metabolism. There are many undiscovered phytocompounds and more than a thousand identified phytocompounds. Although it is well known that plants employ these compounds to protect themselves, recent studies have shown that they can also shield humans from disease [2].

Phenolic compounds, often known as "plant phenols," are a group of plant secondary metabolites that attracted a lot of research interest due to their commercial relevance in textile-, food-, and health-related industries [1]. Plant phenolics exhibit tremendous antioxidant activity and other health benefits. They are regarded as an essential part of the human diet. The phenol moiety and resonance stabilized structure of phenolic acids, a subclass of plant phenolics, give up an H atom, giving them antioxidant properties through a radical scavenging mechanism. The antioxidant action of phenolic acids is also mediated through radical quenching through electron donation and singlet oxygen quenching. Moreover, research on phenolic acids, additional health-protective qualities such as their antibacterial, anticancer, anti-inflammatory, and anti-mutagenic properties is well-documented. The contribution focuses on the potential of phenolic acids in drug development [3].

Ferulic acid (FA), also known as 4-hydroxy-3-methoxycinnamic acid, is a phenolic compound that is frequently present in plant tissues and is mostly found in the primary cell walls of plants [4]. It is typically present in foods such as tomatoes, sweet corn, and rice bran [5]. In response to free radicals, FA demonstrates potent anti-inflammatory effect by donating one hydrogen atom through its phenolic hydroxyl group. Oxidative stress, excessive free radical generation, and hyperglycemia are traits of diabetes. FA exhibits anti-diabetic effects through scavenging the pancreatic free radicals. A significant part of the etiology of cancer is played by free radicals. FA's ability to activate cytoprotective enzymes and scavenge ROS is connected to its anti-carcinogenic effect. Reduced lipid peroxidation, DNA single-strand breakage, inactivation of certain proteins, and disruption of biological membranes are the consequences of this [6]. By reversing the harm produced by nicotine, FA has a beneficial impact on the lungs. It also shields cells from oxidative damage by boosting the body's natural antioxidant defenses. It exhibits antiapoptotic effect by inhibition of externalization of phosphatidyl serine in human peripheral blood mononuclear cells. It also exhibits neuroprotective, radioprotective, and anti-aging effects [5]. Due the numerous therapeutic effects of ferulic acid make it a valuable phytocompound in drug discovery process. Hence, the present study aims to study the molecular mechanism of ferulic acid using network pharmacology approach.

The one-drug/one-target/one-disease approach to drug discovery, which is currently dealing with many issues of safety, efficacy, and sustainability, has recently lost popularity in favor of network biology and polypharmacology approaches for omics data integration and multitarget drug development, respectively. A new paradigm known as network pharmacology (NP), which examines the effects of medications at the interactome and diseasome levels simultaneously, was created

as a result of the fusion of network biology and polypharmacology. To attempt to comprehend the activities and interactions of the drug with multiple targets, this new field has emerged. Using computing power, a systematic catalogue of a drug molecule's molecular interactions in a living cell is produced. In addition to improving both the safety and effectiveness of currently available drugs, NP analysis also enables new therapeutic options [7].

METHODS

PubChem database screening and ADMET analysis

One of the richest libraries of information on chemical compounds and their biological activity is PubChem (https://pubchem.ncbi.nlm.nih.gov) [8]. The chemical formula, 3D structure, and canonical SMILES of ferulic acid were retrieved using this database. ADMET analysis was performed using the canonical SMILES in ADMETlab 2.0. The widely used AMDETlab web server has undergone a thorough redesign to become ADMETlab 2.0, which predicts the pharmacokinetics and toxicity of substances [9].

Target gene prediction using SwissTargetPrediction and STITCH

SwissTargetPrediction and STITCH database was used to retrieve predicted target genes for ferulic acid in Homo sapiens. In STITCH, the filters were set to not more than 50 interactors in the first shell and targets with minimum required interaction score of more than 0.400 were taken into account. SwissTargetPrediction is a free online service that accurately predicts the targets of bioactive chemicals using a combination of 2D and 3D similarity measures with known ligands (http://www.swisstargetprediction.ch) [10]. You can access STITCH (a "search tool for chemical interactions") at http://stitch.embl.de/. It incorporates data on interactions from binding tests, metabolic pathways, crystal structures, and drug-target relationships. The chemical relationship networks can be explored using STITCH, including in the context of associated binding proteins [11]. The target genes obtained from SwissTargetPrediction and STITCH were then used as input in Gene List Venn Diagram (https://www.bioinformatics.org/gvenn/) to check for duplicate target genes and eliminate them.

Protein-protein network construction and analysis using STRING database

You can access the STRING database online at https://string-db.org/. It seeks to include both known and predicted functional and physical interactions between proteins. To do this, the STRING database gathers and evaluates data from a variety of sources, including: The systematic transfer of interaction evidence from one organism to another, databases of interaction experiments and annotated complexes or pathways, and automated text mining from scientific literature and computational interaction predictions based on conserved and co-expression genomic context [12]. The list of 79 target genes obtained using SwissTargetPrediction and STITCH were used here to create a protein-protein interaction network in Homo sapiens and the minimum interaction score of 0.900 was used. Further, k-means clustering was also performed.

Gene enrichment analysis using ShinyGO

The list of 79 target genes obtained using SwissTargetPrediction and STITCH was used in the ShinyGO tool the for KEGG pathway, GO Molecular function, GO biological processes, and disease alliance enrichment analysis to understand the roles of target proteins interacting with ferulic acid. An easy-to-use and graphical tool for enrichment analysis is ShinyGO. With graphical visualization of enrichment, protein interactions, pathway, and gene properties, ShinyGO is used for in-depth analysis of gene list lists. A sizable annotation database generated from the STRING and Ensembl databases serves as the foundation for ShinyGO. One of ShinyGO's distinctive features is its application program interface, which provides access to STRING and KEGG databases for the retrieval of protein-protein interaction networks and pathway diagrams. Another feature is the graphical visualization of enrichment results and gene characteristics [13].

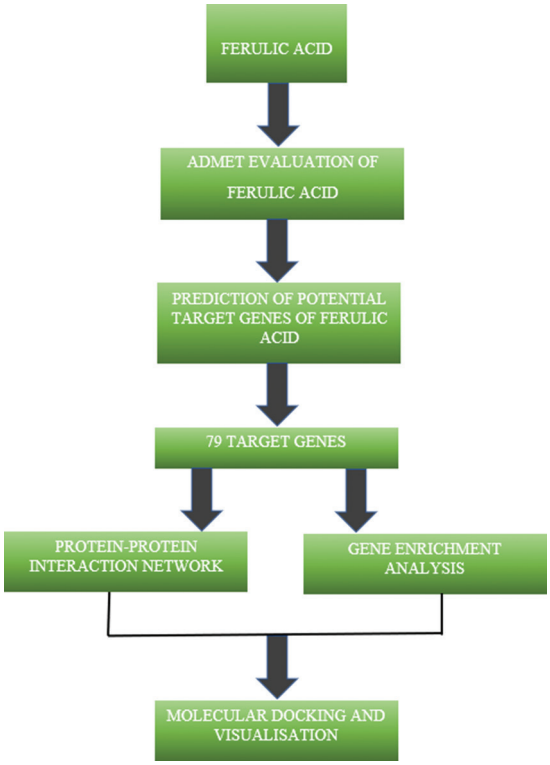

Fig. 1: The workflow of the *in silico* analysis of ferulic acid

Molecular docking

Ligand preparation

The 3D structure of Ferulic acid downloaded in.sdf format from PubChem Database was converted to.pdb format using Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/).

Protein preparation

The protein structures for STAT3, ALOX15, and RELA target genes (PDB ID: 6TLC, 7LAF, and 1NFI) were retrieved from the Protein Data Bank (PDB) database is an international repository for structural information on biological macromolecules [14]. All the structures were then purified using Biovia Discovery Studio Visualizer. Water molecules, hetero atoms, and all chains except the A chain of all proteins were removed and polar hydrogen were added to the structures. The commercial-grade Biovia Discovery studio visualizer is a tool for visualizing, analyzing, and sharing protein, and modeling data [15].

Docking and visualization

Docking of the ligand with all the three proteins was performed using PyRx. PyRx is a program for virtual screening in computational drug discovery that enables screening of libraries of compounds against putative therapeutic targets. It allows medicinal chemists to conduct virtual screening from any platform and supports users at every stage of the procedure, from data preparation through job submission and outcome analysis. PyRx is a useful tool for computer-aided drug design since it has a docking wizard with an intuitive UI. For structure-based drug creation, PyRx additionally contains a potent visualization engine and chemical spreadsheet-like functionality [16]. The protein structures were converted to.pdbqt format and energy minimization was done for the ligand and then converted to.pdbqt format. Docking was performed for ligand with each protein and the docked complex having the lowest binding energy was selected for visualization.

Visualization was performed using Biovia Discovery Studio Visualizer. The docked ligand structure along with purified protein was uploaded

and the 2D and 3D interactions of the same were visualized after labeling the amino acids interacting with ligand and customizing the structures.

RESULTS

Ligand information retrieval and ADMET analysis

The chemical formula, canonical SMILES, and 3D structure of ferulic acid were retrieved from PubChem database as shown in Fig. 2 and Table 1. The ADMET analysis of ferulic acid performed using ADMETlab 2.0 indicated that it falls under acceptable category and consists of all drug-likeness properties. The analysis results are shown in Table 2 and Fig. 3.

Target genes prediction of ferulic acid

SwissTargetPrediction and STITCH were used for prediction of target genes of ferulic acid. The results obtained were a list of 79 genes (Table 3) that are possible targets of ferulic acid. There targets were further used for protein-protein network construction.

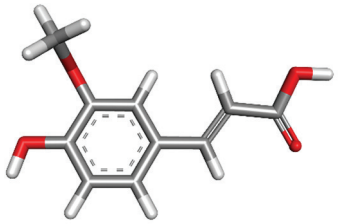

Fig. 2: 3D structure of ferulic acid

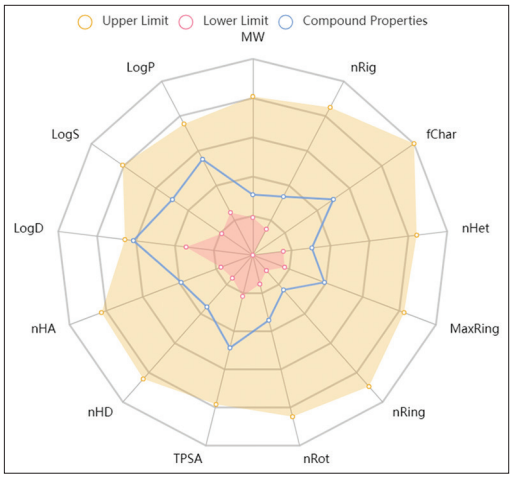

Fig. 3: Bioavailability radar for ferulic acid

Table 1: Ligand name, PubChem ID, molecular formula, and canonical SMILES of ferulic acid

Ligand name	PubChem ID	Chemical formula	Canonical SMILES
Ferulic acid	445,858	$C_{10}H_{10}O_4$	COC1=C (C=CC (=C1) C=CC (=O) O) O

Protein-protein interaction (PPI) network construction

The 79 target genes retrieved from SwissTargetPrediction and STITCH were imported into the STRING database and a protein-protein interaction network was constructed for Homo sapiens and minimum required interaction score of 0.900 was selected. The PPI obtained as shown in Fig. 4 consisted of 79 nodes, 54 edges, an average node degree of 1.37, the average local clustering coefficient of 0.393, and a PPI enrichment p-value of 5.2e-09. Further, after performing k-means clustering, the first cluster (Fig. 5) contained 33 genes, the second cluster (Fig. 7) contained 21 genes, and third cluster (Fig. 7) contained 25 genes. RELA, ALOX15, and STAT3 from cluster 1, cluster 2, and cluster 3, respectively, were found to have maximum interactions with other genes in the cluster and were taken further for molecular docking studies.

Gene enrichment analysis

The list of 79 target genes was imported to ShinyGo tool for gene enrichment analysis. Enrichment carried out for KEGG pathway (Fig. 8) reflected that the target gene proteins are majorly involved in nitrogen metabolism among others. The target proteins in GO molecular function category are involved in carbonate dehydratase activity, hydroperoxy icosatetraenoate dehydratase activity, estrogen 16-alpha-hydroxylase activity, and more (Fig. 9). In the GO biological process category, they are mostly implicated in one-carbon metabolic process, bicarbonate transport, and response to amyloid-beta (Fig. 10). Finally, in the disease alliance category, they are involved in stomach carcinoma, cervix uteri carcinoma *in situ*, pulmonary emphysema, lung disease, and many more as shown in Fig. 11.

Molecular docking and visualization

Molecular docking was performed using PyRx for the three target gene proteins selected after PPI network analysis. RELA (PDB ID: 1NFI), ALOX15 (PDB ID: 7LAF), and STAT3 (PDB ID: 6TLC) were docked with Ferulic acid (PubChem ID: 445858). The docked complex having lowest binding energy as shown in Table 4 was taken further for visualization using Biovia Discovery Studio Visualizer. The results visualization of the 2D and 3D interactions is shown in Figs. 12 and 13, respectively. The non-bond information is shown in Table 5.

DISCUSSION

Over the past 10 years, the rate of drug failure in late-stage clinical development has increased in tandem with the preponderance of the idea that the goal of drug discovery is to create ligands that are as highly selective as possible to work solely on specific therapeutic targets. A congruence between genetic reductionism and emerging molecular biology methods that allowed for the isolation and characterization of specific "disease-causing" genes gave rise to the concept of "one gene, one drug, one disease" or rational drug design. On the other hand, network biology proposes that the approach to drug discovery should be to find the changes in the network that causes the disease. According to network biology study, perturbing robust phenotypes may involve manipulating many proteins as in most situations; there is little impact on illness networks when a single node is deleted. In place of the prevalent presumption of single target drug discovery, a new method to drug discovery known as polypharmacology is emerging with an expanded knowledge of the role of networks in the redundancy and robustness of biological systems issues. This novel method has important effects on the toxicity and efficacy of the drug development process. Network pharmacology thereby expands the existing window

Table 2: Absorption, distribution, metabolism, excretion, and toxicity analysis of ferulic acid

Molecular weight	Absorption		Distribution	Metabolism		Excretion	Toxicity		
	WS	SP	BBB	CYP3A4-sub	CYP2C19-inh	TC	ORAT	HT	AMES
194.06	−1.761		0.329	0.075	0.026	7.48	0.733	0.345	0.114

WS=Water solubility (log mol/L), SP=Skin permeability (log Kp), BBB=Blood brain barrier permeability, TC=Total clearance (log mol/min/kg), HT=Human hepatotoxicity, ORAT=Oral rat acute toxicity

Table 3: Predicted target genes of ferulic acid

Serial number	Gene	Uniprot ID	Description
1	CA2	P00918	Carbonic anhydrase II
2	CA7	P43166	Carbonic anhydrase VII
3	CA1	P00915	Carbonic anhydrase I
4	CA6	P23280	Carbonic anhydrase VI
5	CA12	O43570	Carbonic anhydrase XII
6	CA14	Q9ULX7	Carbonic anhydrase XIV
7	CA9	Q16790	Carbonic anhydrase IX
8	CA5A	P35218	Carbonic anhydrase VA
9	CA5B	Q9Y2D0	Carbonic anhydrase VB
10	MAOB	P27338	Monoamine oxidase B
11	AKR1B1	P15121	Aldose reductase
12	ALOX5	P09917	Arachidonate 5-lipoxygenase
13	MMP9	P14780	Matrix metalloproteinase 9
14	MMP1	P03956	Matrix metalloproteinase 1
15	MMP2	P08253	Matrix metalloproteinase 2
16	PTPN1	P18031	Protein-tyrosine phosphatase 1B
17	CA13	Q8N1Q1	Carbonic anhydrase XIII (by homology)
18	CA3	P07451	Carbonic anhydrase III
19	APP	P05067	Beta amyloid A4 protein
20	NFE2L2	Q16236	Nuclear factor erythroid 2-related factor 2
21	STAT3	P40763	Signal transducer and activator of transcription 3
22	HSD11B1	P28845	11-beta-hydroxysteroid dehydrogenase 1
23	ESR2	Q92731	Estrogen receptor beta
24	CA4	P22748	Carbonic anhydrase IV
25	TLR4	O00206	Toll-like receptor 4 (by homology)
26	PTGS1	P23219	Cyclooxygenase-1
27	MET	P08581	Hepatocyte growth factor receptor
28	CYP1A1	P04798	Cytochrome P450 1A1
29	CYP1A2	P05177	Cytochrome P450 1A2
30	NQO2	P16083	Quinone reductase 2
31	CYP1B1	Q16678	Cytochrome P450 1B1
32	CPA1	P15085	Carboxypeptidase A1
33	EGFR	P00533	Epidermal growth factor receptor erbB1
34	PTGS2	P35354	Cyclooxygenase-2
35	TTR	P02766	Transthyretin
36	KDM4E	B2RXH2	Lysine-specific demethylase 4D-like
37	KDM3A	Q9Y4C1	Lysine-specific demethylase 3A
38	KDM6B	O15054	Lysine-specific demethylase 6B
39	FTO	Q9C0B1	Alpha-ketoglutarate-dependent dioxygenase FTO
40	KDM4A	O75164	Lysine-specific demethylase 4A
41	KDM4C	Q9H3R0	Lysine-specific demethylase 4C
42	TUBB1	Q9H4B7	Tubulin beta-1 chain
43	RELA	Q04206	Nuclear factor NF-kappa-B p65 subunit
44	FYN	P06241	Tyrosine-protein kinase FYN
45	LCK	P06239	Tyrosine-protein kinase LCK
46	SLC16A1	P53985	Monocarboxylate transporter 1 (by homology)
47	TLR9	Q9NR96	TLR7/TLR9
48	AKR1B10	O60218	Aldo-keto reductase family 1 member B10
49	ALOX15	P16050	Arachidonate 15-lipoxygenase
50	PRKCE	Q02156	Protein kinase C epsilon
51	F3	P13726	Coagulation factor VII/tissue factor
52	NOS2	P35228	Nitric oxide synthase, inducible
53	NGFR	P08138	Low affinity neurotrophin receptor p75NTR
54	CCND1	P24385	Cyclin-dependent
	CDK4	P11802	kinase 4/cyclin D1
55	TUBB3	Q13509	Tubulin beta-3 chain
56	ABCB1	P08183	P-glycoprotein 1
57	FBP1	P09467	Fructose-1, 6-bisphosphatase
58	TOP2A	P11388	DNA topoisomerase II alpha
59	GLO1	Q04760	Glyoxalase I
60	BACE1	P56817	Beta-secretase 1
61	ACE	P12821	Angiotensin-converting enzyme (by homology)
62	REN	P00797	Renin
63	PARP1	P09874	Poly (ADP-ribose) polymerase-1
64	MAOA	P21397	Monoamine oxidase A
65	AHR	P35869	Aryl hydrocarbon receptor
66	KDM2A	Q9Y2K7	Lysine-specific demethylase 2A
67	TPMT	P51580	Thiopurine S-methyltransferase
68	CTNNB1	P35222	Axin1/beta-catenin
69	F2	P00734	Thrombin
70	SLC13A5	Q86YT5	Solute carrier family 13 member 5
71	CYCS	P99999	Cytochrome c, somatic
72	ERVFRD-1	P60508	Endogenous retrovirus group FRD, member 1
73	CXCL2	P19875	Chemokine (C-X-C motif) ligand 2
74	CXCL3	P19876	Chemokine (C-X-C motif) ligand 3
75	CXCL1	P09341	Chemokine (C-X-C motif) ligand 1
76	TMEM30B	Q3MIR4	Transmembrane protein 30B
77	TMEM30A	Q9NV96	Transmembrane protein 30A
78	ERVW-1	Q9UQF0	Endogenous retrovirus group W, member 1
79	TMEM30C	A0ZSE6	Transmembrane protein 30C

TLR: Toll-like receptor

of opportunity for druggable targets [17]. The present study was done using this concept of network pharmacology.

The chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) plays important roles in drug discovery and development together with network pharmacology. A drug candidate should have adequate ADMET qualities at a specific therapeutic dose in addition to sufficient efficacy against the therapeutic target. The "Rule of Five," which was developed by Lipinski and his colleagues, is a well-known rule-based drug-likeness filter that determines whether a molecule is effectively absorbed orally or not. Molecular weight (MW) ≤ 500, octanol/water partition coefficient (A log P) ≤ 5, the quantity of hydrogen bond donors (HBDs) ≤ 5, and the quantity of hydrogen bond acceptors (HBAs) ≤ 10, are the five rules. If a molecule breaks two or more of the four requirements, it would not be considered orally active, according to this criterion [18]. Based on these rules, ferulic acid is orally active. Along with Lipinski rule of five, in the present study, the drug-likeliness of ferulic acid is supported by the forementioned results. In the absorption studies, logS that is the logarithm of aqueous solubility value was studied and ferulic acid showed optimal absorption. The blood-brain barrier (BBB) penetration was taken into account, which is essential for medications that act on the CNS and need to cross the BBB to reach their molecular target. Ferulic acid demonstrated adequate BBB penetration. Almost two-thirds of known medicines in humans are metabolized by the 57 isozymes that make up the human cytochrome P450 family (phase I enzymes), with five isozymes accounting for the majority of this activity (A2, 3A4, 2C9, 2C19, and 2D6). In the metabolic study of ferulic acid, CYP3A4 and CYP2C19 isoforms were taken into account and results showed probability of being substrate and inhibitor, respectively. Clearance (CL), a crucial pharmacokinetic parameter that determines, along with the volume of distribution, the half-life and, consequently, how frequently the medication should be provided, was investigated in the excretion investigations. Ferulic acid

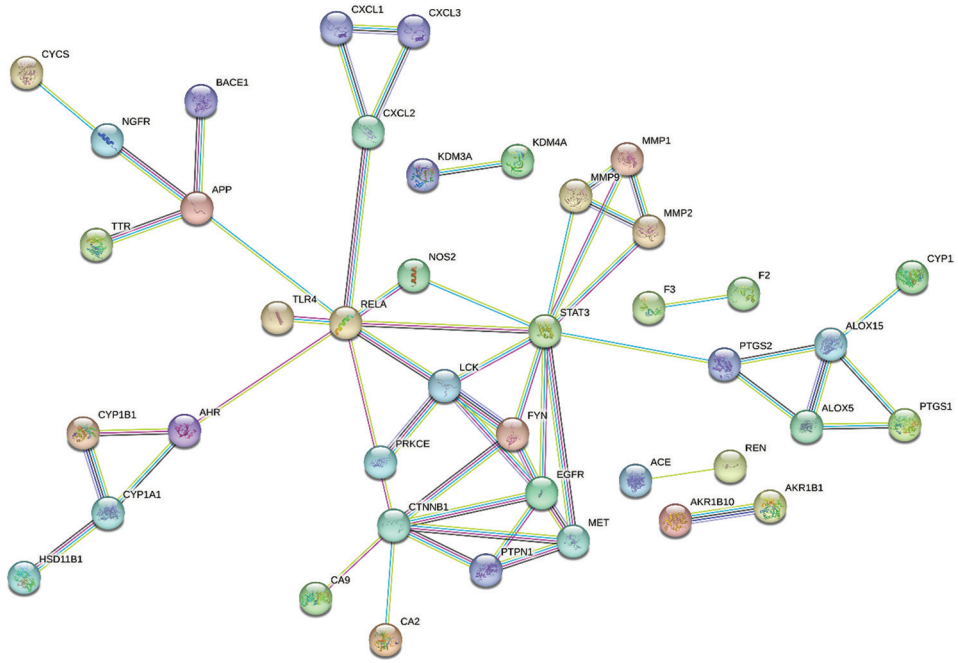

Fig. 4: PPI network obtained with minimum required interaction score of 0.900

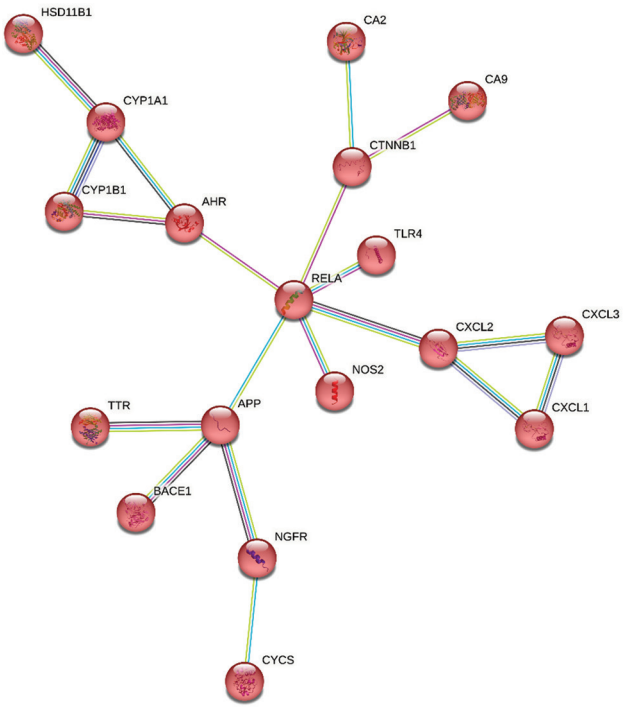

Fig. 5: Cluster 1 after performing k-means clustering

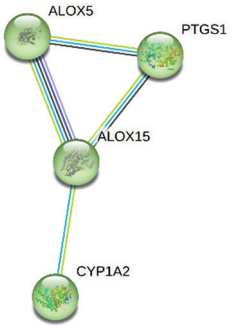

Fig. 6: Cluster 2 after performing k-means clustering

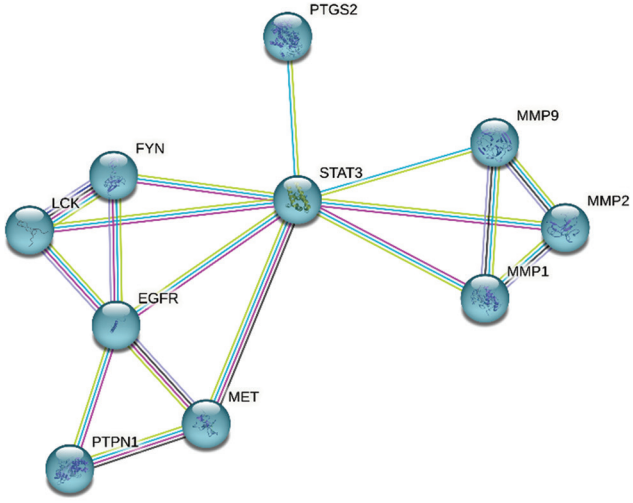

Fig. 7: Cluster 3 after performing k-means clustering

showed a moderate clearance value. In the toxicity studies, the human hepatotoxicity (HT), the AMES test for mutagenicity and Rat Oral Acute Toxicity (ORAT) which is determination of acute toxicity in mammals were studied. Ferulic acid showed no toxicity in the HT and AMES test [19].

One of the important aspects of drug discovery using network pharmacology is identification of multiple target genes for the drug candidate. In this study, the target genes for ferulic acid were identified using SwissTargetPrediction and STITCH database which resulted in 79 possible target genes (Table 3). There genes were then used to construct PPI network using STRING database followed by k-means clustering which generated three clusters. On the analysis of the clusters generated, one gene having maximum number of interactors

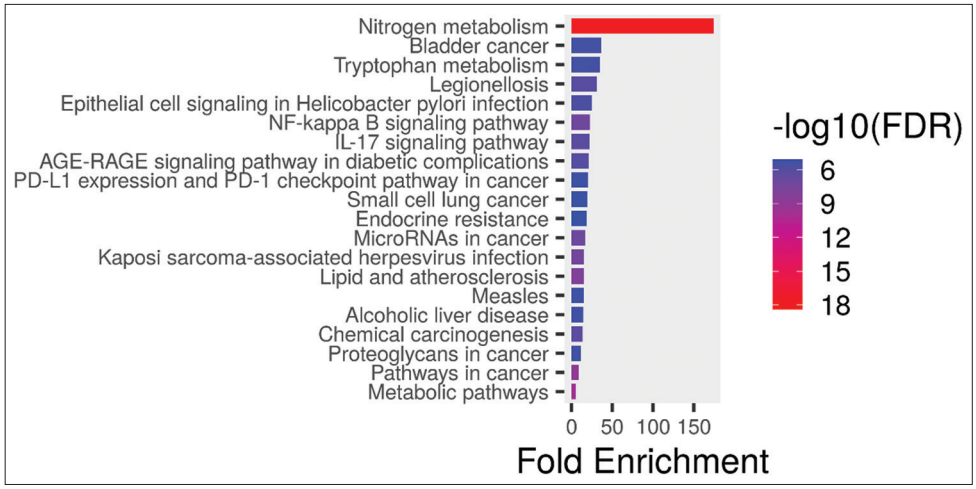

Fig. 8: KEGG pathway enrichment

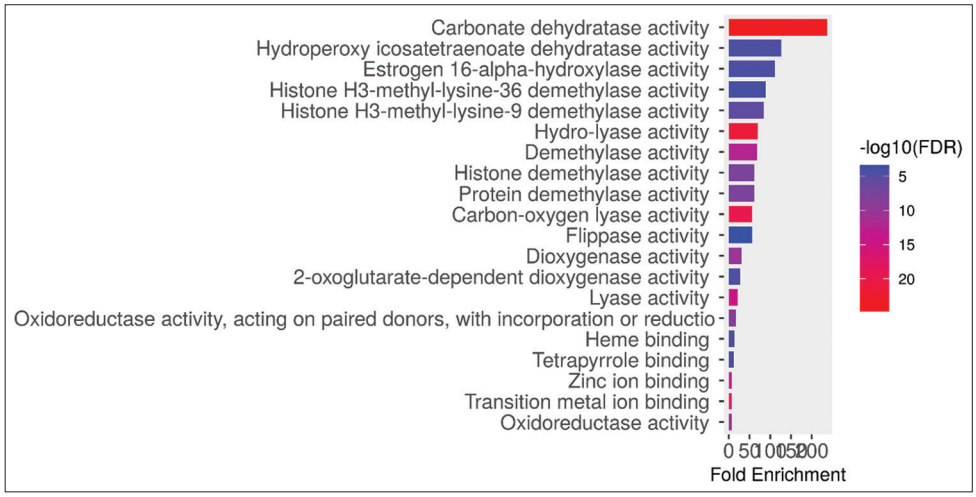

Fig. 9: GO Molecular function enrichment

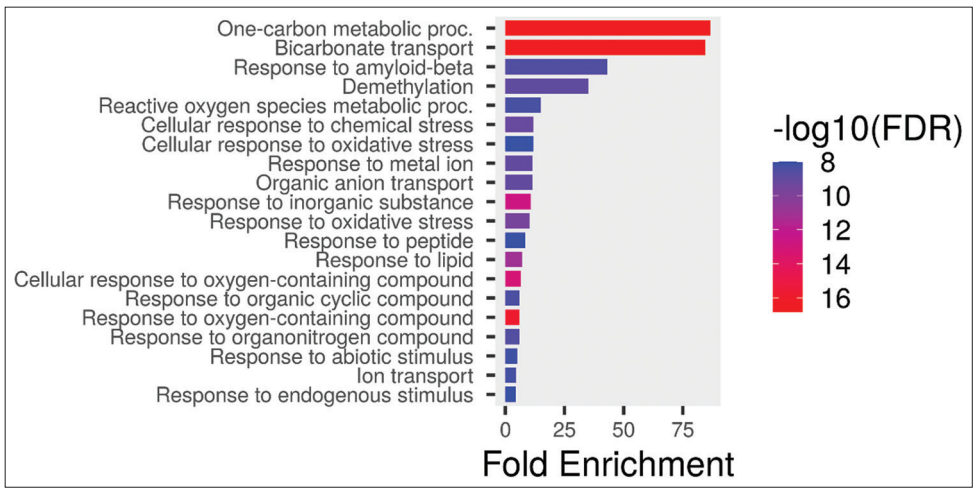

Fig. 10: GO biological process enrichment

was selected from each cluster (RELA, ALOX15, and STAT3). These were further taken up for molecular docking with Ferulic acid to study the ligand-protein interaction. All three docked structures showed low binding energy which suggests that ferulic acid may interact with these targets. Among the three targets, ALOX15 required least binding energy suggesting significant interacting with ferulic acid. These results suggest that ferulic acid may show pharmacological activity against these possible targets.

The list of target genes obtained from SwissTargetPrediction and STITCH database were to perform gene enrichment analysis using ShinyGo tool. The KEGG pathway analysis showed that the

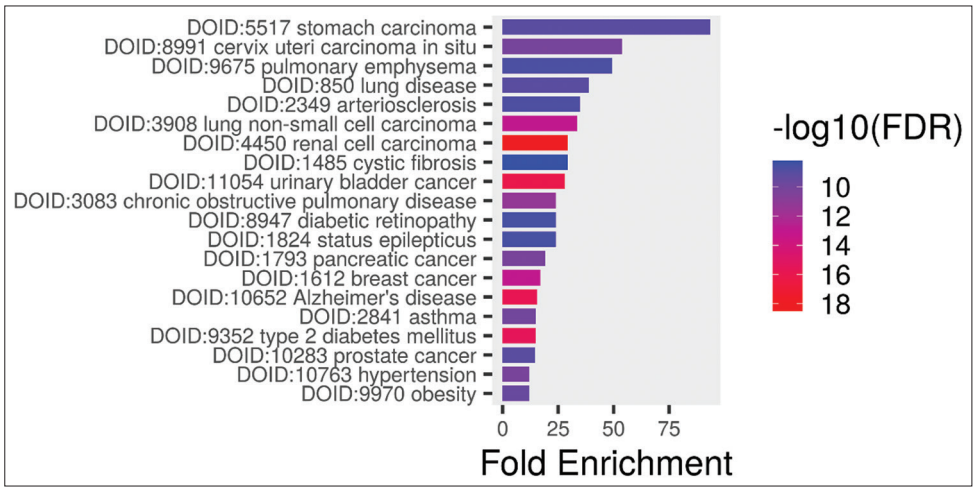

Fig. 11: GO disease alliance enrichment

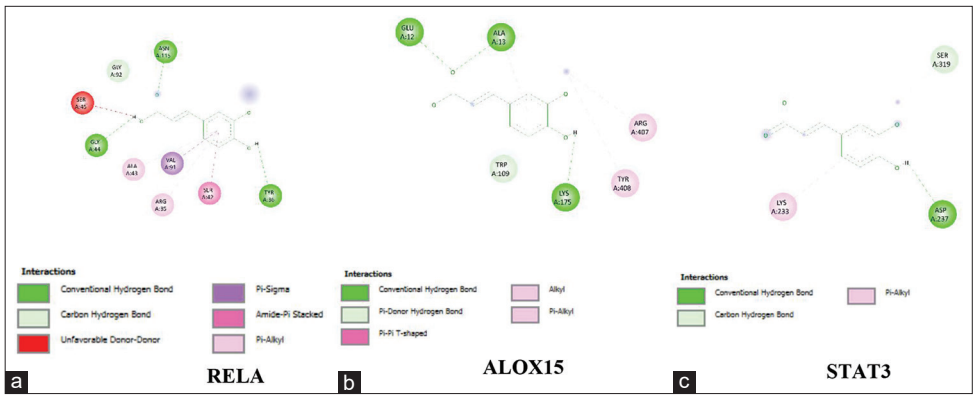

Fig. 12: 2D interaction visualisation of gene target proteins with Ferulic acid. (a) RELA, (b) ALOX15, (c) STAT3

Table 4: Binding affinity of target gene protein with ferulic acid

Ligand	Binding affinity		
	RELA (1NFI)	ALOX15 (7LAF)	STAT3 (6TLC)
Ferulic acid	−5.8	−6.5	−6.0

Table 5: Nonbond information

Name	Bonds	From	To
1nfi-Ferulic acid	Hydrogen bond	A: ASN115:HD21	N: UNK0:O
	Hydrogen bond	N: UNK0:H	A: GLY44:O
	Hydrogen bond	N: UNK0:H	A: TYR36:O
	Hydrogen bond	A: GLY92:CA	N: UNK0:O
	Hydrogen bond	A: VAL91:CG2	N: UNK0
	Hydrogen bond	A: SER42:C, O; ALA43:N	N: UNK0
	Hydrogen bond	N: UNK0	A: ARG35
	Hydrogen bond	N: UNK0	A: ALA43
6tlc-Ferulic acid	Hydrogen bond	N: UNK0:H	A: ASP237:OD1
	Hydrogen bond	N: UNK0:C	A: SER319:OG
	Hydrophobic bond	N: UNK0	A: LYS233
7laf-Ferulic acid	Hydrogen bond	A: GLU12:HN	N: UNK0:O
	Hydrogen bond	A: ALA13:HN	N: UNK0:O
	Hydrogen bond	N: UNK0:H	A: LYS175:O
	Hydrogen bond	A: TRP109:HE1	N: UNK0
	Hydrophobic	A: TRP109	N: UNK0
	Hydrophobic	N: UNK0:C	A: ARG407
	Hydrophobic	A: TYR408	N: UNK0:C
	Hydrophobic	N: UNK0	A: ALA13

possible target genes of ferulic acid are majorly involved in nitrogen metabolism. The metabolism of nitrogen affects how malignancies form. According to one study, a malfunction in nitrogen metabolism may use the Wnt signaling pathway to hasten the development of lung adenocarcinoma [20]. Target genes may also be involved in tryptophan metabolism, according to the pathway analysis. Several neurological illnesses (Alzheimer's disease, autism, Parkinson's disease, Huntington's disease, epilepsy, amyotrophic lateral sclerosis, and multiple sclerosis) and psychiatric problems (Depression, Anxiety, Schizophrenia, and Bipolar disorder) are very strongly tied to different enzymes or products of the tryptophan metabolic process [21]. This suggests that ferulic acid may be a candidate drug of cancer, neurological disorders and psychiatric disorders. The GO molecular function analysis results implicated the involvement of target genes in Carbonate dehydratase activity, hydroperoxy icosatetraenoate dehydratase activity, Estrogen 16-alpha-hydroxylase activity, and Histone H3-methyl-lysine-36 demethylase activity. There are studies that suggest association of Carbonate dehydratase activity with sleep apnea severity and related Hypoxemia [22]. Estrogen metabolites created by Estrogen 16-alpha-hydroxylase activity, biologically strong estrogens, are associated with breast cancer risk [23]. The Go biological process analysis showed involvement of target genes in mainly in One-carbon metabolic process, bicarbonate transport, and response to amyloid-beta. One carbon metabolism and bicarbonate transport are known to be associated with cancer [24,25]. Other illnesses include brain dysfunction; kidney stones, systemic acidosis, and hypertension are brought on by defective bicarbonate transport. Bicarbonate transporter expression levels have been found to be altered in patients with lung, breast, and colon cancer [25]. In case of response to amyloid-beta, genetic variation in the response to amyloid beta- deposition is observed to influence

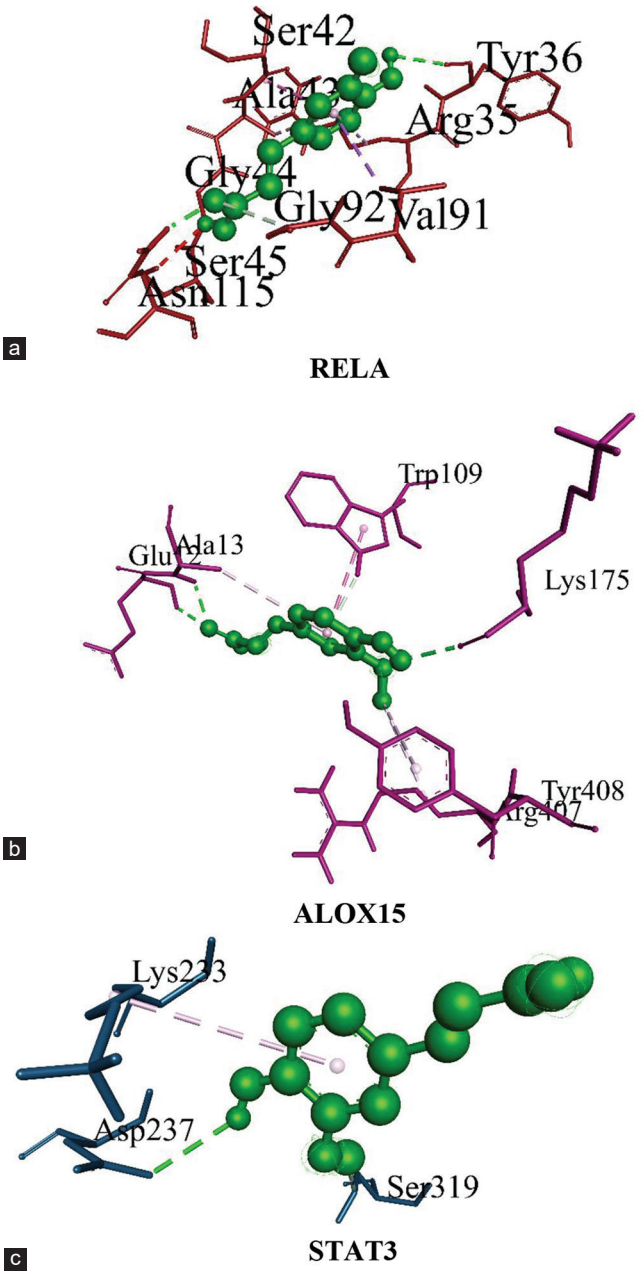

Fig. 13: 3D interaction visualisation of gene target proteins with ferulic acid. (a) RELA, (b) ALOX15, (c) STAT3

the risk of Alzheimer's disease [26]. The disease alliance analysis revealed stomach carcinoma, cervix uteri carcinoma *in situ*, pulmonary emphysema, lung disease, and arteriosclerosis as major diseases associated with the target genes. These findings suggest that ferulic acid may have a broad range of pharmacological effects and may be a possible drug candidate against these diseases or disorders.

ALOX15 gene encodes an enzyme that is a member of the lipoxygenase family of proteins. This enzyme acts on various polyunsaturated fatty acid substrates generating many bioactive lipid mediators such as lipoxins, eicosanoids, hepoxilins, and other molecules. The encoded enzyme and its reaction products have been associated with regulating immunity and inflammation [27]. This gene has been implicated with most diseases mentioned earlier such as inflammation, vascular diseases such as hypertension, atherosclerosis, neurological diseases such as Alzheimer's disease, Parkinson's disease, and also other diseases such as diabetes and obesity [28]. The PPI network analysis showed that ALOX15 was one of the gene with maximum number of interactors and molecular docking studies showed that it required least binding to interact with ferulic acid, these results also strongly support that ferulic acid may be a candidate drug against these diseases.

CONCLUSION

Ferulic acid may have a variety of pharmacological effects, according to the results of this research study based on network pharmacology and molecular docking. The information from the gene enrichment analysis can be utilized to develop ferulic acid as a drug that is effective in treating a variety of conditions, including cancer, neurological disorders, psychiatric disorders, Alzheimer's disease, pulmonary emphysema, arteriosclerosis, and many more. This study offers a fresh viewpoint on the use of ferulic acid as a treatment for the conditions indicated above.

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