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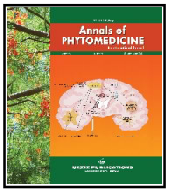

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Bioinformatics approach to identify molecular targets of chrysin against Alzheimer's disease

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Abstract

Chrysin is a natural compound with numerous pharmacological properties, especially antioxidant, anti-inflammatory, antiviral, antitumor, anticancer, and hepatoprotective activities. Although, the biological activities of chrysin have been described and its possible pharmacological properties have been previously determined, its pharmacokinetic properties against Alzheimer's disease (AD) have not been fully elucidated based on gene targets, drug-likeness, molecular signaling pathways, and network-based pharmacology analyses. In this study, we aimed to reveal the molecular targets and potential interactions of chrysin against AD by gene-set enrichment and bioinformatics approach. The chrysin was entered into the PubChem and ChEBI database, and the targets of chrysin were estimated using DIGEP-Pred. Then, GeneCards, DisGeNET, PharmGKB, and Swiss Target Prediction were used to identify possible interacting genes and proteins. The drug-likeness properties and toxicity characteristics of chrysin were determined using Swiss ADME and ProToxII databases. In addition, STRING and KEGG enrichment database were used to elucidate the role of probable interacting proteins to construct a protein-protein interaction (PPI) network and a network of molecular targeting pathways, respectively. Based on the results of pharmacokinetic properties and drug-likeness analysis, chrysin predicted to have a good drug-likeness activity (score = – 0.21), as well as good brain barrier permeability (BBB score = 3.71) with no observable toxicity. A total of 38 genes were identified as the top genes that interact with chrysin against Alzheimer's disease. ILB, IL6, TNF, MAPK1, CASP3, PSEN1, PSEN2, PTGS2, NFKB1, AKT1, GSK3B, and APP were selected as top core targets that may play a significant role in AD treatment. Furthermore, a total of 158 different pathways were identified as the probably modulated pathways, corresponding to 38 protein targets. Besides neurodegeneration and AD, pathways in cancer, lipid and atherosclerosis, EGFR tyrosine kinase inhibitor resistance, AGE-RAGE signaling in diabetic complications, HIF-1 signaling, PI3K-Akt signaling, MAPK signaling, IL-17 signaling, neurotrophin and sphingolipid signaling were defined as the top pathways associated with chrysin-regulated proteins. Overall, the results indicated that the network-based approach could provide a novel approach to uncover the therapeutic mechanisms of chrysin against AD.

1. Introduction

Chrysin (5,7-dihydroxyflavone) is a natural flavonoid found in propolis, honey, fruits, and passion flowers. It is mainly present in the species of *Pleurotus ostreatus, Oroxylum indicum, Matricaria chamomilla*, *Passiflora incarnata* and *P. caerulea*.This natural compound has been studied for its potential pharmacological properties and it has been reported to have various pharmacological properties, such as antioxidant, anti-inflammatory, antiviral, antitumor, anticancer, antihyperlipidemic, antidepressant, and antibacterial activities (Mani and Natesan, 2018). Chrysin has been found to increase the therapeutic efficacy of docetaxel and mitigate docetaxel-induced edema. It has also been shown to target myeloid-derived suppressor cells and enhance tumor response to anti-PD-1 immunotherapy (Stompor-Goracy, 2021; Li *et al*., 2022). Chrysin induces apoptosis in cancer cells by activating caspase-3 and PLC-λ1 degradation, downregulating XIAP and inactivating Akt (Khoo *et al*., 2010). Additionally, chrysin has been reported to have anti-inflammatory effects by downregulating the key pro-inflammatory enzymes, inducible nitric oxide synthase (iNOS) and cyclooxy-genase-2 (COX-2) (Zeinali *et al*., 2017). Chrysin has also been found to possess cardioprotective activity by improving post-ischemic functional recovery and suppressing vascular endothelial growth factor (VEGF)-induced angiogenesis (Kasala *et al*., 2015). Chrysin also shows promise as an anxiolytic and neuroprotective agent, with some studies indicating its potential for improving cognitive function and memory. Additionally, chrysin has been shown to have antidiabetic effects, reducing blood glucose levels and improving insulin sensitivity. Its diverse range of potential therapeutic applications has made chrysin an area of interest in the field of natural medicine and drug discovery (Satyanarayana *et al*., 2015; Shooshtari *et al*., 2020).

Alzheimer's disease (AD) is a complex neurodegenerative disease that is attributed to a combination of multiple factors, *e.g*., synaptic dysfunctions such as synapses loss, deficits in synaptic plasticity,

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senile dementia, and progressive disability. These dysfunctions are thought to be extremely associated with cognitive decline in Alzheimer's disease patient. The disease leads to disruption of processes vital to neurons and their networks, including communication, metabolism, and repair. It has been observed that patients with AD typically lose neurons and connections in memory-related parts of the brain, including the entorhinal cortex and hippocampus (Sun *et al*., 2017; Gezici and Sekeroglu, 2022; Ju and Tam, 2022). Alzheimer's disease is characterized by changes in the brain that result in the loss of neurons and their connections, including the development of amyloid plaques and neurofibrillary, or tau, tangles. These changes affect a person's ability to remember and think and, eventually, to live independently. The main causes of AD and other neurodegenerative diseases are aggregated protein accumulation and oxidative damage (Calderon-Garcidueñas and Duyckaerts, 2018; Espay *et al*., 2019). Secondary metabolites of plants, such as terpenoids and flavonoids, have been shown to possess biological activities that can be useful in the prevention and treatment of AD. Terpenoids have been found to possess antioxidant activity and the potential to increase the level of acetylcholine, which is important for cognitive function. Flavonoids have been found to inhibit acetylcholinesterase, butyrylcholinesterase, Tau protein aggregation, β-secretase, oxidative stress, inflammation, and apoptosis through modulation of signaling pathways implicated in cognitive and neuroprotective functions. Additionally, natural compounds found in various parts of the medicinal plants and/or marine sources may potentially protect against neurodegeneration alongside improve memory and cognitive function. Even though, a numerous studies have been performed toreveal biological activities and pharmacological properties of chrysin, network-based molecular and pharmacological activities of chrysin have not been performed until now (Akram and Nawaz, 2017; Gezici and Sekeroglu, 2019; Sekeroglu and Gezici, 2019; Singh *et al*., 2021; Choudhir *et al*., 2022; Wu *et al*., 2022). Therefore, we aimed to identify the molecular targets and potential interactions of chrysin against AD by gene-set enrichment and bioinformatics approach. This research could provide a novel approach to uncover the therapeutic mechanisms of chrysin against AD.

2. Materials and Methods

2.1 Chemical compositions and predicted targets

Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used for dictionary of molecular entities and chemical properties of chrysin (Hastings *et al*., 2016). The targets of chrysin were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on structural formula of chrysin (Lagunin *et al*., 2013).

2.2 Pharmacokinetic properties and drug likeness analysis

PubChem database was used to obtain chemical structure and pharmacological properties of chrysin, as well as Chemical Entities of Biological Interest (ChEBI) database. Swiss ADME and ProToxII were used to determine drug likeness possibilities and toxicity properties of chrysin, respectively (Daina *et al*., 2017; Banerjee *et al*., 2018).

2.3 Prediction of targets by gene set enrichment analysis

GeneCards, The Human Gene Database, was used to evaluate probable interacting genes of chrysin. Based on this database, top interacting genes were analyzed using unique GeneCards identifiers (GC ids), provided by the GeneLoc Algorithm (Harel *et al*., 2009; Fishilevich *et al*., 2016). DisGeNET (version 7.0) database and the pharmacogenomics knowledge base (Pharm GKB) were employed to reveal the data about disease associated genes and variants from multiple sources (Thorn *et al*., 2013; Pinero *et al*., 2020). Additionally, SMILES into Swiss Target Prediction, a network-based tool for target prediction of bioactive molecules that were used to predict all the chrysin-related targets (Daina *et al*., 2019).

2.4 Construction protein-protein interaction (PPI) network

STRING database was used to annotate the role of probable interacting genes and proteins associated with chrysin. PPI network mapping was conducted on chrysin and protein targets using the retrieval of interacting genes database with the species limited to "homo sapiens" and a confidence score ≥ 0.4 (Wu *et al*., 2009; Athanasios *et al*., 2017).

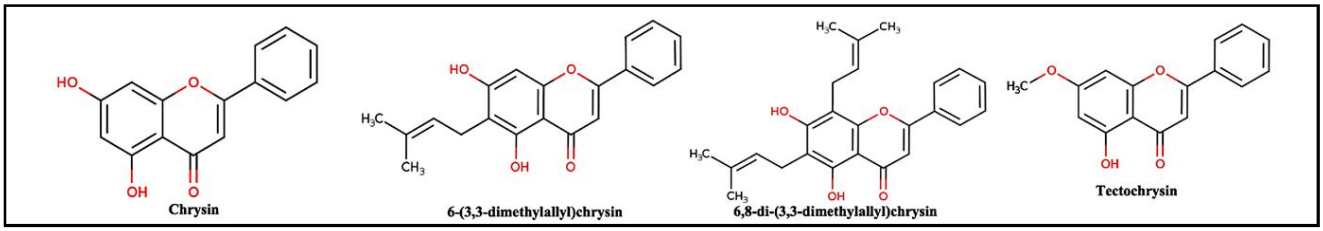
2.5 KEGG enrichment analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is an integrated database of genes and genomes used for mapping pathways at molecular level. KEGG enrichment analysis was performed for construction the network regulated by chrysin (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa *et al*., 2017).

3. Results

3.1 Results of chemical compositions and predicted targets

Chrysin ($C_{15}H_{10}O_4$) belonging to the class of flavone, includes a dihydroxyflavone in which the two hydroxy groups are located at positions C-5 and C-7. Similar to other flavones, chrysin is a yellow crystalline secondary compound soluble in water and ethanol. It is mainly found in the passion flowers of *Passiflora incarnata* and *P. caerulea*. The synonyms of chrysin with an average molecular mass of 254.237 g/molare chrysin, 5,7-dihydroxy-2-phenyl-4H-1-benzopyran-4-one, 5,7-dihydroxy-2-phenyl-4H-benzo(b)pyran-4-one, 5,7-dihydroxy-2-phenylchromen-4-one, and 5,7-dihydroxy flavone. 3-O-methyl-8 prenylgalangin ($C_{21}H_{20}O_5$), 6-(3,3-dimethylallyl)chrysin ($C_{20}H_{18}O_4$), 6-geranylchrysin ($C_{25}H_{26}O_4$), 6,8-di-(3,3-dimethylallyl) chrysin ($C_{25}H_{26}O_4$), 8-(3,3- dimethylallyl) chrysin ($C_{20}H_{18}O_4$), 8-geranylchrysin ($C_{25}H_{26}O_4$), chrysin 5-xyloside ($C_{20}H_{18}O_8$, chrysin 5,7-dimethyl ether ($C_{17}H_{14}O_4$), chrysin 7-[rhamnosyl-(1->4)-glucoside ($C_{27}H_{30}O_{13}$), chrysin 7-4''-acetylglucoside ($C_{23}H_{22}O_{10}$), chrysin 7-glucoronide ($C_{21}H_{18}O_{10}$), chrysin-7-*O*-glucoronide ($C_{21}H_{18}O_{10}$), chrysin-7-*O*-glucuronide ($C_{21}H_{18}O_{10}$), dimethylstrobochrysin ($C_{18}H_{16}O_4$), helichrysin ($C_{22}H_{24}O_{10}$), rheochrysin ($C_{22}H_{22}O_{10}$), strobochrysin ($C_{16}H_{12}O_4$), and tectochrysin ($C_{16}H_{12}O_4$) are derivatives of chrysin. The chemical structure of chrysin and its derivatives were presented in the Figure 1.



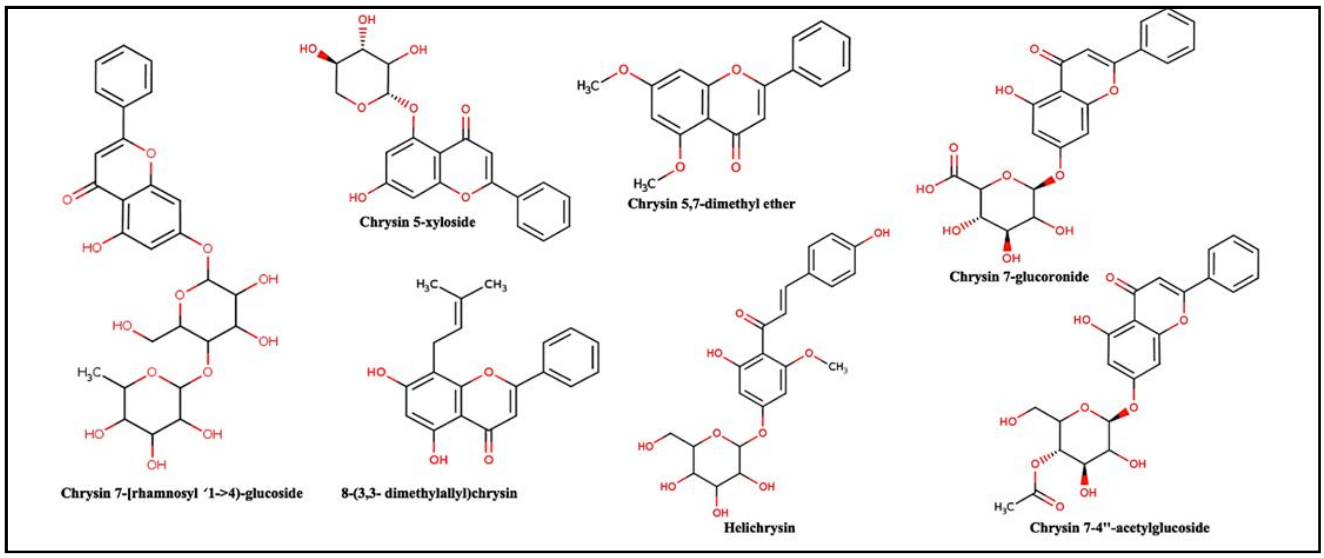


Figure 1: Chemical compositions of chrysin and some derivatives of chrysin.

The targets of chrysin were determined based on prediction of drug-induced changes of gene expression profile for proteins at the pharmacological activity (Pa)>0.7. The findings were given in the Table 1 in which Pa (probability to be active) means the chance that chrysin, whereas Pi (probability to be inactive) means the chance that chrysin is belonging to the subclass of inactive compounds. According to the data presented in the table, chrysin has quite active biological activities including myosin-light-chain kinase inhibitor, anti-inflammatory, antineoplastic, antimutagenic, vasoprotector, and hepatoprotective. Actually, chlordecone reductase inhibitor, membrane integrity agonist, membrane permeability inhibitor, and kinase inhibitor were defined as the most valuable properties of chrysin (Pa>0.7).

Table 1: Prediction of drug-induced changes of gene expression profile for chrysin at pharmacological activity

Pa	Pi	Activity
0,967	0,002	Chlordecone reductase inhibitor
0,965	0,003	Membrane integrity agonist
0,962	0,003	HIF1A expression inhibitor
0,946	0,002	Membrane permeability inhibitor
0,944	0,002	2-Dehydropantoate 2-reductase inhibitor
0,942	0,002	Kinase inhibitor
0,942	0,005	CYP2C12 substrate
0,938	0,001	Aryl-alcohol dehydrogenase (NADP+) inhibitor
0,934	0,003	Anaphylatoxin receptor antagonist
0,933	0,003	Aldehyde oxidase inhibitor
0,928	0,001	P-benzoquinone reductase (NADPH) inhibitor
0,922	0,002	Peroxidase inhibitor
0,916	0,002	Histidine kinase inhibitor
0,915	0,002	Antimutagenic
0,913	0,001	Quercetin 2,3-dioxygenase inhibitor
0,906	0,002	CYP1A inducer
0,906	0,002	NADPH-ferrihemoprotein reductase inhibitor
0,902	0,005	TP53 expression enhancer
0,896	0,003	HMOX1 expression enhancer
0,894	0,003	Vasoprotector
0,896	0,006	Ubiquinol-cytochrome-c reductase inhibitor
0,890	0,002	Alcohol dehydrogenase (NADP+) inhibitor
0,885	0,001	Glycerol dehydrogenase (NADP+) inhibitor
0,885	0,003	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0,885	0,005	CYP1A substrate
0,882	0,003	Cholesterol 26-monooxygenase inhibitor
0,877	0,002	CYP1A1 inducer
0,877	0,002	Beta-carotene 15,15'-monooxygenase inhibitor
0,879	0,006	Antiseborrheic
0,875	0,003	4-Nitrophenol 2-monooxygenase inhibitor
0,874	0,003	UGT1A9 substrate
0,883	0,012	Aspulvinonedimethylallyl transferase inhibitor
0,871	0,001	2-Dehydropantolactone reductase (A-specific) inhibitor
0,872	0,004	CYP1A1 substrate
0,863	0,002	2-Enoate reductase inhibitor
0,853	0,004	UGT1A6 substrate
0,846	0,002	Cystathionine beta-synthase inhibitor
0,842	0,003	MAP kinase stimulant
0,842	0,005	Apoptosis agonist
0,836	0,002	Antihemorrhagic
0,821	0,005	Alkane 1-monooxygenase inhibitor
0,815	0,003	AR expression inhibitor
0,808	0,003	UGT1A3 substrate
0,806	0,004	APOA1 expression enhancer
0,803	0,005	UGT1A substrate
0,799	0,004	CYP1A inhibitor
0,798	0,004	CYP2B5 substrate
0,793	0,003	Monophenol monooxygenase inhibitor
0,795	0,009	JAK2 expression inhibitor

0,793	0,007	UDP-glucuronosyltransferase substrate
0,787	0,002	NADPH oxidase inhibitor
0,788	0,004	Histamine release inhibitor
0,786	0,003	Leukotriene-B4 20-monooxygenase inhibitor
0,799	0,019	Mucomembranous protector
0,774	0,002	1-Alkylglycerophosphocholine O-acetyltransferase inhibitor
0,779	0,008	CYP3A4 inducer
0,774	0,004	CYP1A2 inhibitor
0,773	0,004	UGT1A1 substrate
0,778	0,012	Dehydro-L-gulonate decarboxylase inhibitor
0,767	0,003	UGT1A7 substrate
0,766	0,004	MMP9 expression inhibitor
0,760	0,004	Tetrahydroxynaphthalene reductase inhibitor
0,761	0,006	CYP1A2 substrate
0,758	0,003	Beta glucuronidase inhibitor
0,759	0,004	CYP2A4 substrate
0,756	0,002	CYP1A1 inhibitor
0,769	0,016	Antineoplastic
0,757	0,004	Pectate lyase inhibitor
0,776	0,027	CYP2J substrate
0,778	0,031	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,746	0,002	CF transmembrane conductance regulator agonist
0,748	0,005	Pin1 inhibitor
0,746	0,004	Xenobiotic-transporting ATPase inhibitor
0,741	0,002	CYP19A1 expression inhibitor
0,747	0,009	CYP3A inducer
0,748	0,012	Glutathione thiolesterase inhibitor
0,755	0,021	CYP2J2 substrate
0,738	0,004	Nitrite reductase [NAD(P)H] inhibitor
0,735	0,002	Alcohol dehydrogenase [NAD(P)+] inhibitor
0,732	0,005	Insulysin inhibitor
0,727	0,003	UGT1A10 substrate
0,729	0,006	Ecdysone 20-monooxygenase inhibitor
0,734	0,015	Glucan endo-1,6-beta-glucosidase inhibitor
0,721	0,005	5 Hydroxytryptamine release inhibitor
0,719	0,009	CYP2A6 substrate
0,713	0,003	CYP1B substrate
0,711	0,002	GABA C receptor antagonist
0,707	0,002	Iodide peroxidase inhibitor
0,708	0,004	Antioxidant
0,706	0,003	UGT2B15 substrate
0,705	0,003	NOS2 expression inhibitor
0,710	0,011	NAD(P)+-arginine ADP-ribosyltrans ferase inhibitor
0,701	0,005	UGT2B12 substrate
0,704	0,010	Thioredoxin inhibitor
0,706	0,013	Nitrate reductase (cytochrome) inhibitor

3.2 Results of pharmacokinetic properties and drug-likeness analysis

The chemical and molecular information of chrysin obtained from ChEBI and PubChem, and the relevant drug-likeness properties obtained from SwissADME were summarized in the Table 2. Swiss ADME predicted pharmacokinetic of the chrysin, including topological polar surface area and Lipinski's Rule of 5 recommendations were shown in the Table 2.

Table 2: Pharmacological properties and drug-likeness results of chrysin

ID	ChEBI: 75095
Name	Chrysin
Synonyms	Chrysine, 5,7-Dihydroxy-2-phenyl-4H-1-benzopyran-4-one, 5,7-Dihydroxy-2-phenyl-4H-benzo(b)pyran-4-one, 5,7-dihydroxy-2-phenylchromen-4-one, and 5,7-Dihydroxyflavone
Formula	$C_{15}H_{10}O_4$
Net Charge	0
MW	254.23750 g/mol
Monoisotopic Mass	254.05791 g/mol
Hdon	2
Hacc	4
Rbon	1
TPSA (+!²)	70.67
Melting/boiling point	285.5 °C / 491.91 °C at 760.00 mm Hg (est)
WS	0.105 mg/ml
InChI	InChI=1S/C15H10O4/c16-10-6-11(17)15-12(18)8-13(19-14(15)7-10)9-4-2-1-3-5-9/h1-8,16-17H
SMILES	Oc1cc(O)c2c(c1)oc(cc2=O)-c1ccccc1
Canonical SMILES	C1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O
DL score	-0.21
Lipinski	Yes
Bioavailability score	0.55
Glabsorpsion	High
Coco-2 Permeability	-4.69
BBB permeant	Yes
$T_{1/2}$	0.787
CL	5.131
MolLogP	3.67
MolLogS	-3.51 (in Log(moles/l)) 79.10 (in mg/l)
MolPSA	55.95 A^2
Log K_p (cm/s)	-5.35
Top chemical roles	Radical scavenger and antioxidant activity
Top biological roles	Plant metabolite, myosin-light-chain kinase inhibitor, anti-inflammatory agent, anti-neoplastic agent, and hepatoprotective agent

BBB = blood-brain barrier, DL = drug-likeness, WS = water solubility, GI = gastrointestinal absorption, Hacc = hydrogen bond acceptors, Hdon = hydrogen bond donors, MW = molecular weight, Rbon = rotatable bonds, TPSA = topological polar surface area, CL = clearance.

As shown in the Figure 2, chrysin predicted to possess a good drug-likeness activity with the score of – 0.21, as well as good brain barrier permeability (BBB score = 3.71). In addition, LogP, one of the important components of Lipinski's Rule of 5, was determined as 3.67 that means chrysin can be as an oral drug. *In silico* drug-likeness possibilities of chrysin are given in the Figure 2.

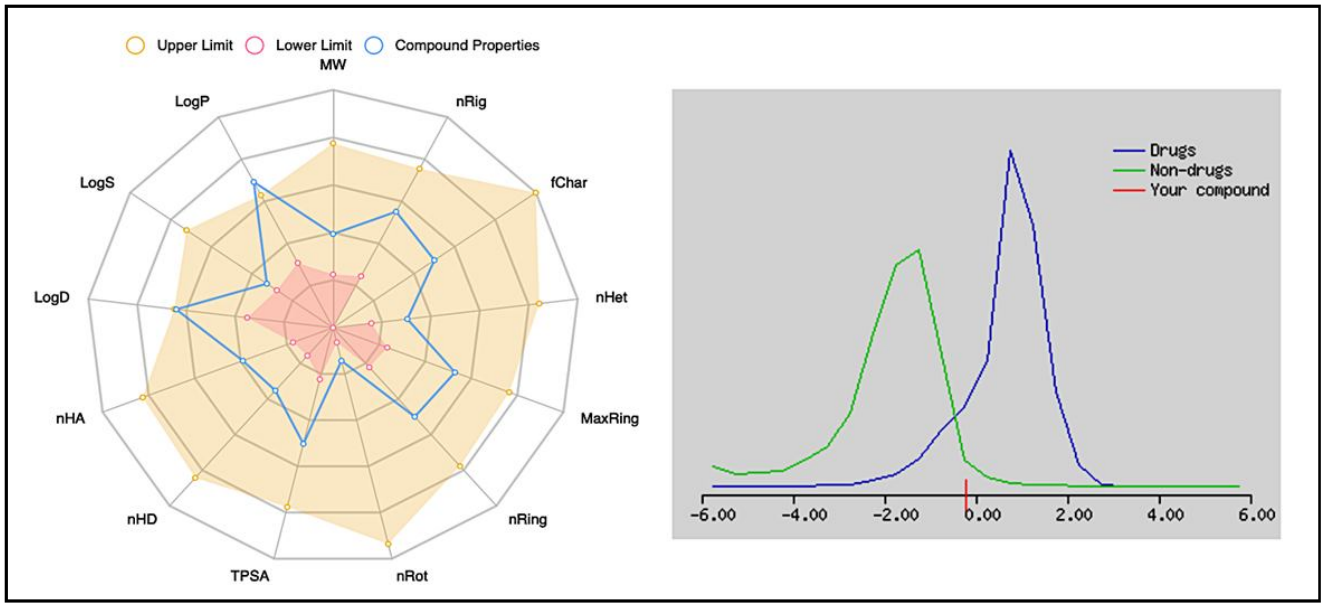


Figure 2: *In silico* drug-likeness properties of chrysin.

In silico toxicological parameters of chrysin were evaluated using ProTox-II software, and the results are presented in Table 3. Oral toxicity prediction results were determined as LD_{50} (lethal $dose_{50}$) values in 3919 mg/kg body weight and the predicted toxicity class of chrysin was 5 according to the globally harmonized system of classification of labelling of chemicals. Furthermore, the results revealed that chrysin showed no observable toxicity, including carcinogenicity, cytotoxicity, hepatotoxicity, immunotoxicity, and mutagenicity. Table 3 also showed that chrysin is a component involved in Tox21-nuclear receptor signaling and Tox21-stress response pathways targeting various ligands.

Table 3: Toxicity prediction report including targets of chrysin

Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.68
Toxicity end points	Carcinogenicity	Inactive	0.62
Toxicity end points	Immunotoxicity	Inactive	0.99
Toxicity end points	Mutagenicity	Inactive	0.57
Toxicity end points	Cytotoxicity	Inactive	0.87
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor (AhR)	Active	1.0
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	Inactive	0.99
Tox21-Nuclear receptor signaling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	Inactive	1.0
Tox21-Nuclear receptor signaling pathways	Aromatase	Active	0.61
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Alpha (ER)	Active	1.0
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Active	1.0
Tox21-Nuclear receptor signaling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	Active	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	Active	1.0
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	Active	1.0
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Active	0.96

3.3 Results of top gene enrichment analysis

In this research, the gene targets of chrysin against AD were collected using GeneCards, DisGeNET, PharmGKB, and SMILES into SwissTargetPrediction. Based on the results, 133 targets were collected to determine the chrysin-associated targets, and 2542 targets were collected Alzheimer disease-related targets. Accordingly, a total of thirty-eight genes were identified as the intersection targets. The detailed information about the targets of chrysin against AD was shown in Table 4.

Table 4: The list of top genes interacts with chrysin against AD

Gene Symbol	Unipot Gene Id	Protein Description
ABCB1	P08183	ATP-dependent translocase
ABCC1	P33527	Multidrug resistance-associated protein-1
ABCG2	Q9UNQ0	ATP-binding cassette transporter
ACHE	P22303	Acetylcholinesterase
AKT1	P31749	AKT serine/threonine kinase 1
APP	P05067	Amyloid-beta precursor protein
AR	P10275	Androgen receptor
BAX	Q07812	Apoptosis regulator BAX
BCHE	P06276	Butyrylcholinesterase
BCL2	P10415	Apoptosis regulator Bcl-2
BDNF	P23560	Brain-derived neurotrophic factor
CASP3	P42574	Caspase 3
CLU	P10909	Clusterin
ECE1	P42892	Endothelin converting enzyme 1
EGFR	P00533	Epidermal growth factor receptor
ESR1	P03372	Estrogen receptor 1
ESR2	Q92731	Estrogen receptor 2
GSK3B	P49841	Glycogen synthase kinase 3 beta
HMOX1	P09601	Heme oxygenase 1
IGF1	P05019	Insulin-like growth factor
IL17A	Q16552	Interleukin 17A
IL1B	P01584	Interleukin 1 beta
IL6	P05231	Interleukin 6
MAOA	Q5ULA9	Monoamine oxidase A
MAPK1	P28482	Mitogen-activated protein kinase
MET	P08581	MET proto-oncogene, receptor tyrosine kinase
MPO	P05164	Myeloperoxidase
NFKB1	P19838	Nuclear factor kappa B subunit 1
NOS3	P29474	Nitric oxide synthase, endothelial
PLAU	Q03405	Plasminogen activator, urinary
PLD2	O14939	Phospholipase D2
PPARG	P37231	Peroxisome proliferator activated receptor gamma
PSEN1	P49768	Presenilin 2
PSEN2	P49810	Presenilin 2
PTGS2	P35354	Prostaglandin G/H synthase 2
TNF	P01375	Tumor necrose factor
TP53	P04637	Tumor protein p53
VEGFA	P15692	Vascular endothelial growth factor A

3.4 Results of protein – protein interaction network

The relationship of a total of 38 proteins between each other were constructed from STRING database with PPI enrichment p-value< 1.0e-16 (FDR < 0.05). This enrichment value means that these proteins have more interactions among themselves than what would be expected for a random set of proteins of the same size and degree distribution drawn from the genome. PPI network was presented in the Figure.

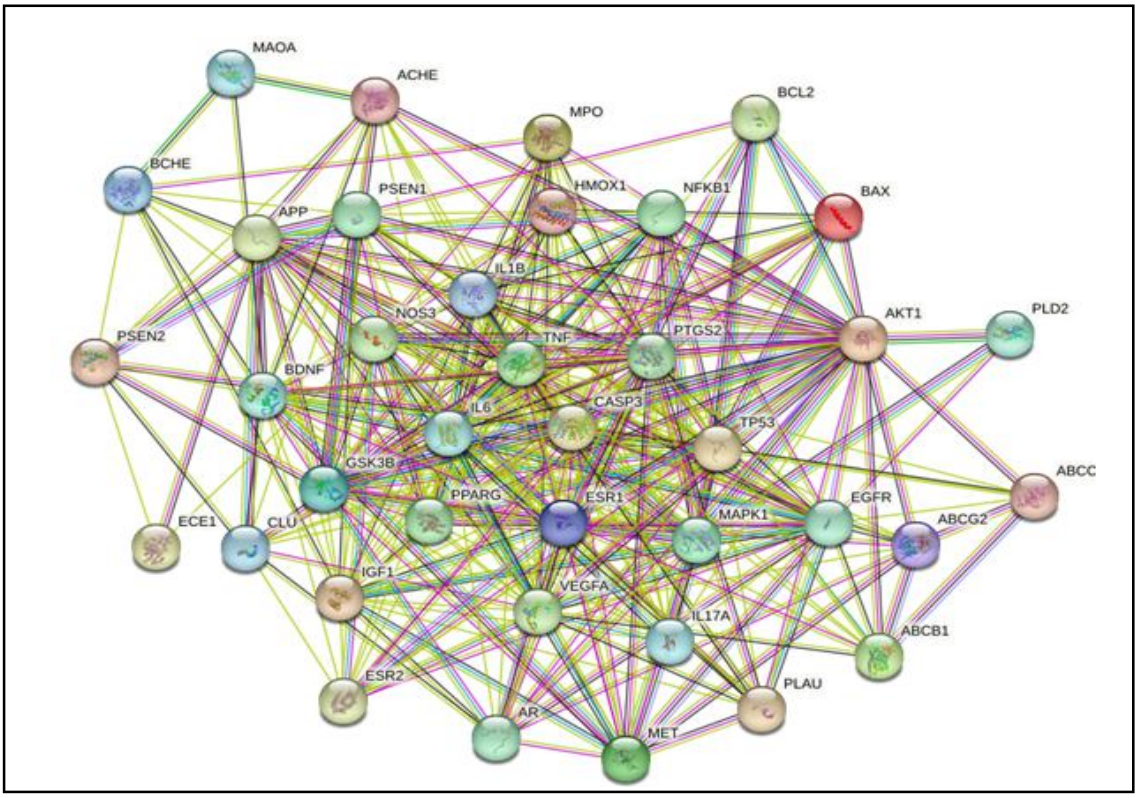


Figure 3: Protein-protein interaction networks of chrysin against AD-related targets.

As can be seen in the Figure, CASP, ESR1, MAPK1, NOS3, IL1B, VEGFA, and PTGS2 are the proteins that located in the center of the network. ILB, IL6, TNF, MAPK1, CASP3, PSEN1, PSEN2, PTGS2, NFKB1, AKT1, GSK3B, and APP were selected as top core targets that may play a significant role in AD treatment.

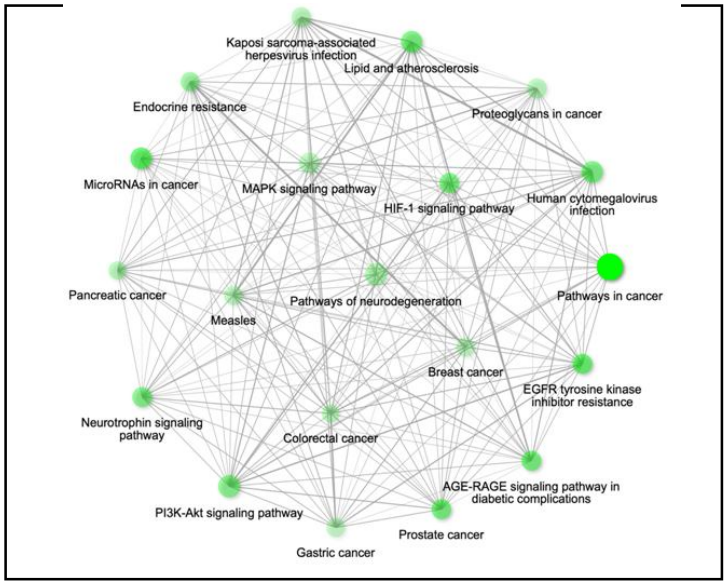


Figure 4: Network of top related pathways construction with KEGG enrichment.

3.5 Results of KEGG enrichment pathway

According to the KEGG enrichment pathway analyses, a total of 158 distinct pathways were identified as the probably modulated pathways by chrysin. A network corresponding to 38 protein targets are schematized in Figure 4, summarizing the correlations between the major pathways listed in the enrichment network. As presented in the Figure 4, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways.

Accordingly, pathways in cancer, microRNAs in cancer, lipid and atherosclerosis, hypoxia-inducible factor 1 (HIF-1) signaling pathway, PI3K-Akt signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance, advanced glycation endproducts-receptor for advanced glycation endproducts (AGE-RAGE) signaling in diabetic complications, neurotrophin signaling pathway, sphingolipid signaling pathway, interleukin-17 (IL-17) signaling pathway, neurodegeneration, and Alzheimer's disease were determined as the top pathways associated with chrysin-regulated proteins with the lowest false discovery rate (FDR<0.05) (Figure 4).

4. Discussion

Network-based pharmacology is a promising approach for identifying effective phytochemicals against various diseases, including Alzheimer disease. This approach involves exploring the biological function, protein-phytochemical/drugs network, and up-down regulation of pathological host target proteins to understand the mechanism of phytochemicals against the disease. Network pharmacology combines phytochemical information with bioinformatics tools to illustrate herbal formulae holistically in the context of phytochemical basis and therapeutic mechanisms. Studies have used network pharmacology to investigate significant phytochemicals, pathways, and targets against AD and to explore the molecular evidence of herbal formulae. Accordingly, this approach can be used to identify potential targets and efficacy prediction to uncover the therapeutic effect of herbal drugs (Boezio *et al*., 2017; Dragomir *et al*., 2018). Although, biological effects and pharma-cological profiling of chrysin have been studied, network-based genomic targets of chrysin, molecular signaling pathways and pharmacokinetic properties of chrysin have not been revealed until now (Akram and Nawaz, 2017; Gezici and Sekeroglu, 2019; Sekeroglu and Gezici, 2019; Singh *et al*., 2021; Choudhir *et al*., 2022; Wu *et al*., 2022). In this context, this research was aimed to investigate the molecular targets and potential interactions of chrysin against AD by gene-set enrichment and bioinformatics approach.

In the current research, we first identified the pharmacokinetic properties and toxicity of chrysin via electronic databases. Chrysin compliance with Lipinski rule of 5, and with a higher LD_{50} value. The literature has previously reported oral administration of chrysin (5000 mg/kg) showed 40% mortalityin acute oral toxicity, while daily oral administration of chrysin (1000 mg/kg) showed significantly decreased body weight and significantly increased liver weight in male rats in the sub-chronic toxicity study (Yao *et al*., 2023), as consistent with the estimated results of this work. In other research aimed to investigate toxicity of chrysin by acute and sub-chronic oral toxicity in rats, LD_{50} value estimated for the chrysin was 4350 mg/kg, in fact, chrysin caused 40% of dead's in both male and female rats at the dose of 5000 mg/kg body weight (Falbo and Aiello, 2023), which is close to the results of this bioinformatic research.

Recent studies have focused on the role of chrysin in targeting specific genes involved in Alzheimer's disease, such as the amyloid-beta precursor protein (APP). Chrysin has been found to protect against the accumulation of beta-amyloid plaques in the brain, which are a hallmark of Alzheimer's disease. Additionally, chrysin has been shown to have anti-inflammatory and antioxidant properties that may help mitigate the effects of neurodegeneration (Calderon-Garcidueñas and Duyckaerts, 2018; Ju and Tam, 2022).Therefore, chrysin-associated targets and Alzheimer disease-related targets were determined in the present research. This research showed that chrysin regulates the activities of genes related to AD in a way that causes an increase in the expression levels of some of the genes, *e.g*., antioxidant molecules, while it causes a decrease in others, *e.g*., IL-1β, IL-10 and TNF-α. Further research is needed to fully understand the potential benefits of chrysin in treating Alzheimer's disease, but early studies show promise for this natural compound as a potential therapeutic option. Liu *et al.* (2021) investigated the "hidden" multi-target strategy of novel chrysinderivatives in combination with its molecular targets for the treatment of Alzheimer's disease. Amongst the synthesis derivatives, compound 3 was found as a potential hidden multifunctional candidate in the therapy of AD, thanks to its good ADMET (absorption, distribution, metabolism, excretion and toxicity) score (Liu *et al*., 2021). Nonetheless, a recent meta-analysis of genome-wide association studies on Alzheimer's disease and related dementias identified new loci and enabled the generation of a new genetic risk score associated with the risk of Alzheimer's disease (Bellenguez *et al*., 2022).

Afterwards, PPI analyses of targeting proteins were conducted and CASP, ESR1, MAPK1, NOS3, IL1B, VEGFA, and PTGS2 are identified as the core proteins that located in the center of the network.In addition, target signaling pathways modulated by chrysin were determined using KEGG enrichment in this research. It is well-known that multiple signaling pathways interact with each other in the metabolic processes in living organisms. Based on the pathway analyses, most of the genes regulated by chrysin are found to closely associate with cancer, microRNAs in cancer, HIF-1 signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance, AGE-RAGE signaling in diabetic complications, neurotrophin signaling pathway, sphingolipid signaling pathway, IL-17 signaling pathway, neurodegeneration, and Alzheimer's disease. ILB, IL6, TNF, MAPK1, CASP3, PSEN1, PSEN2, PTGS2, NFKB1, AKT1, GSK3B, and APP genes are involved in Alzheimer's disease. In agreement with the findings from this network-based research, previous reports indicated that chrysin is used to synaptic dysfunctions such as synapses loss, deficits in synaptic plasticity, senile dementia, and progressive disability, which are closely associated with the development of AD (Akram and Nawaz, 2017; Calderon-Garcidueñas and Duyckaerts, 2018; Espay *et al*., 2019; Ju and Tam, 2022; Wu *et al*., 2022).

5. Conclusion

Alzheimer's disease (AD) is a complex neurodegenerative disease that is attributed to a combination of multiple factors, including synaptic dysfunctions, deficits in synaptic plasticity, senile dementia, and progressive disability. It is characterized by changes in the brain that result in the loss of neurons and their connections, including the

development of amyloid plaques and neurofibrillary, or tau, tangles. The main causes of AD and other neurodegenerative diseases are aggregated protein accumulation and oxidative damage. Phytochemicals such as terpenoids and flavonoids, have been found to possess biological activities. In particularly, have been found to possess antioxidant activity and the potential to increase the level of acetylcholine, while flavonoids have been found to inhibit acetylcholinesterase, butyrylcholinesterase, Tau protein aggregation, β-secretase, oxidative stress, inflammation, and Alzheimer's disease. Chrysin is a natural flavonoid that possess remarkable biological and pharmacological activities in the human metabolism. The results showed that the network-based techniques could provide a new approach to discover the mechanisms of chrysin forthe prevention and treatment of AD. Even though it is rare to use chrysin alone as a medicine nowadays, it is expected that chrysin will be most likely to be used in the future drug discovery. Further studies, especially clinical trials are necessary to confirm the pharmacokinetic properties and molecular targets of chrysin against AD.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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