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Review Article

Exploring the Neuroprotective Potential of Puerarin in Alzheimer's Diseases: 'A Network Pharmacology and Molecular Docking Approach'

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Abstract

Alzheimer's disease is the most common neurodegenerative disorder in the central nervous system. As Herbal medicines are mainly used as a complementary and alternative therapy. This study aimed at investigating the potential therapeutic effects, potential Alzheimer associated targets, and underlying molecular mechanisms of Formononetin in the treatment of Alzheimer's disease. Puerarin-associated potential targets, and metabolic pathways involved in Puerarin disease were explored through systemic biology-based network pharmacology assay and molecular docking Chemo informative tools and databases i.e. DIGEP-Pred and DisGeNET were used. Further, STRING was used to enrich the protein-protein interaction for the bioactive modulated targets. Similarly, molecular docking was performed using AutoDock Vina. In the following network pharmacology assay, bioactives in camellia sinesis were identified based on druglike characteristics, and pharmacokinetics profiles. Moreover, forty-four molecular targets and fifteen biochemical pathways were uncovered through molecular docking. TNF, AKT1, EGFR, TP53, APTGS2, BCL2, PPARG, ESR1 ,HIF1A and MMP9 are implicated in the inhibition of Alzheimer's symptoms through modulating the cAMP signaling pathway, calcium signaling pathway, GABAergic and dopaminergic synaptic activities, pathway. In terms of safety, efficacy, and sustainability, the one-drug/one-target/one-disease approach to drug discovery is currently experiencing numerous difficulties. Recently, network biology and polypharmacology approaches have become more popular as methodologies for multitarget drug development and omics data integration, respectively. These two methods were combined to produce a brand-new paradigm known as network pharmacology (NP), which examines how medications affect both the interactome and the diseasome level.

Keywords: Alzheimer's Disease; Isoflavonoids; Puerarin; Network Pharmacology; Molecular Docking

Introduction

Alzheimer's disease

Alzheimer's disease is a chronic neurodegenerative disorder that affects memory, thinking, and behaviour, and progresses over time through different stages. It primarily affects the elderly population. The word "dementia," which means "observable decline in mental abilities," comes from the Latin word "de mens." A decline in cognitive, affective, and conative elements of mental functioning characterises this acquired clinical condition. The main cause of dementia is Alzheimer's Disease which is a progressive neurodegenerative disorder which affects memory, thinking and behaviour and is one of the greatest healthcare challenges of the 21st century [1]

Alzheimer's disease is a chronic condition with 20-year-long preclinical and early phases and an 8-10-year normal clinical course. The disease is thought to have a prevalence of 10-30% and an incidence of 1-3% in people over 65 years of age [3]. The disease accounts for 50-60% of all cases. The prevalence of dementia is below 1% in individuals aged 60-64 years, but shows an almost exponential increase with age, so that in people aged 85 years or older the prevalence is between 24% and 33% in the Western world [16]. There are various stages of AD which include the Preclinical Stage, Mild Cognitive Impairment Stage (MCI), mild, moderate and severe dementia stages [13]. The disease results in cognitive, functional, and behavioural changes and the signs and symptoms include aggressive behaviour, psychomotor agitation, wandering, changes in personality difficulty with routine tasks, decreased or poor judgment, impaired visuospatial skills, disorientation, memory loss, difficulty with problem-solving and decision-making, depression and sleep disturbances [14]. In the brain, a protein is produced that results in the formation of plaques and tangles. Microscopically, neurotic plaques producing Aβ-42 and neurofibrillary tangles (NFTs) made of hyperphosphorylated tau are signs of AD. These proteins are the starting points for the breakdown of connections between nerve cells, which will eventually lead to the death of nerve cells and the destruction of brain tissue [19].

Isoflavonoids

Isoflavonoids are a type of natural product that belongs to a group of plant secondary metabolites with a polyphenolic structure that can be found in a variety of fruits, vegetables, and drinks. They have a variety of beneficial biochemical and antioxidant properties that have been linked to diseases like cancer, Alzheimer's disease (AD), atherosclerosis, and others. Flavonoids are an essential component in a number of nutraceutical, pharmacological, medical, and cosmetic uses because they have a wide range of health- promoting benefits. [19,24]. The ability of isoflavonoids to interact with signaling pathways, as well as their antioxidant and metal-chelating characteristics, may contribute to their therapeutic potential for halting the onset and progression of AD. Although supplementing with flavonoids may improve cognitive performance, the potential usefulness of flavonoids is primarily limited to the prevention period or to the early stages of the development of the disease. This is because metal-induced oxidative stress in AD primarily affects cognition and short and long-term memory [22].

Only plants can produce flavonoids, and those that are present in animals or people are descended from plants. Flavonoids themselves are divided into several subclasses, including anthocyanins (such as cyanidin), flavonols (such as epigallocatechin gallate, procyanidin, and teaflavins), flavanones (such as hesperidin, naringenins, and neohesperidines), flavones (such as apigenin, diosmetin, and luteolin), flavonos (e.g. Daizein, Genistein). The primary form in which they are present is as aglycones, the fundamental building blocks of flavonoids, glycosides, and methylated derivatives. Apyrone (flavones and flavonols) or a dihydro derivative of it can make up the six-member ring attached to benzene rings. The most intriguing pharmacological properties of flavonoids are their ability to chelate metal ions or scavenge free radicals, which is mostly dependent on their functional hydroxyl group [15].

Network pharmacology

Network pharmacology is a relatively new field that aims to understand the complex interactions between drugs, targets, and disease networks using a systems-level approach. This approach integrates a wide range of computational and experimental methods to analyse complex networks and identify key drug targets and pathways. One of the main advantages of network pharmacology is its ability to identify potential drug targets and pathways that may not be readily apparent using traditional approaches. Network pharmacology studies have identified novel targets for cancer and neurodegenerative diseases that were previously unknown [11]. In addition, network pharmacology approaches can be used to predict drug efficacy, toxicity, and drug-drug interactions. Another important application of network pharmacology is in the development of personalized medicine. By analysing the unique genetic, epigenetic, and environmental factors that contribute to disease, network pharmacology can identify drugs and drug combinations that are most likely to be effective for a particular patient. This approach has been particularly successful in cancer research, where network pharmacology has been used to develop targeted therapies that are tailored to the specific genetic mutations of individual patients. One of the challenges in network pharmacology research is the complexity of the networks themselves. Drug-target-disease networks are highly interconnected, with multiple feedback loops and cross-talk between different pathways. As a result, network pharmacology approaches require sophisticated algorithms and data integration techniques to accurately analyse and interpret the data. In addition, network pharmacology studies require large datasets and exten-

sive computational resources, which can be a significant barrier to entry for many researchers. Network pharmacology is a rapidly growing field that has the potential to revolutionize drug discovery and development. By using a systems-level approach to analyse complex drug target-disease networks, network pharmacology can identify novel drug targets, predict drug efficacy and toxicity, and develop personalized therapies [2,12].

Application of network pharmacology in AD

Network pharmacology, an emerging interdisciplinary field, has gained increasing attention in recent years for its potential in understanding the complex mechanisms underlying AD and discovering new therapeutic strategies. Network pharmacology employs computational and systems biology approaches to investigate the interactions among multiple components of a biological system, including genes, proteins, metabolites, and pathways, in a holistic manner. By integrating diverse data sources, such as omics data, drug databases, and network analysis, network pharmacology enables a comprehensive and systematic understanding of the molecular mechanisms of AD and the identification of potential drug candidates. Several studies have demonstrated the application of network pharmacology in AD research. Network pharmacology-based approach to uncover the molecular mechanisms of Chinese herbal medicines in treating AD [15], and identified potential drug targets and active components using network analysis. The authors used a network pharmacology-based strategy to build an AD-associated target network by integrating various sources of biological data, including target genes, protein-protein interactions, and disease associated pathways. Moreover, network pharmacology has been employed to identify novel drug candidates for AD treatment. A developed a chemometric method based on network pharmacology to predict the oral bioavailability of compounds and identified potential AD drug candidates with favourable pharmacokinetic properties [19]. The application of network pharmacology in AD has several advantages. It allows researchers to identify potential drug targets that may not be apparent through traditional approaches, as it considers the network context and interconnectedness of molecules. Network pharmacology can also help predict the effects of drugs on multiple targets simultaneously, enabling the identification of drug combinations that may have synergistic effects or targeting multiple pathways involved in AD pathogenesis [23].

Isoflavonoids in network pharmacology

Because of their potential health benefits, flavonoids-a broad collection of naturally occurring chemicals that are prevalent in fruits, vegetables, tea, and other plant-based foods-have received a lot of attention. These bioactive compounds are well-known for their anti-inflammatory, antioxidant, and anticancer and heart-protective properties. Because the intricate processes that unerlie isoflavonoids' beneficial effects entail interactions with several targets and signaling pathways, they are prime candidates for network pharmacology research [28]. The way that flavonoids work in the human body involves modifying a complex system of molecular interactions. They regulate many physiological processes and signaling cascades through interactions with enzymes, receptors, transporters, and transcription factors. By charting gene expression patterns, drug-target relationships, and protein-protein interactions networks, network pharmacology offers a systems-level comprehension of the mechanisms by which isoflavonoids carry out their medicinal actions. Through the mapping of the interactions between isoflavonoids and medication targets, scientists are able to determine possible combinations that could improve treatment outcomes or lessen adverse effects. This method may completely change the process of finding new drugs and make it easier to create cutting-edge therapeutic approaches. Comprehending these interplays can aid in the creation of customized and focused methods for illness prevention and management [5].

Network pharmacology of Puerarin in Alzheimer's disease

Puerarin is a member of flavonoid phytoestrogens, which is the main active ingredient of *red clover*, a leguminous plant. In BV2 mouse microglia stimulated by LPS, Puerarin significantly reduced TNF-α, IL-1β and IL-6 by inhibiting the NF-κB signal pathway and the production of COX2, PGE2 and iNOS. In vivo, Puerarin demonstrated a dose-dependent inhibition of TNF-α and IL-1β in the hippocampus of mice induced by high-fat diet. The most likely mechanism was to inhibit the proinflammatory NF-κB signal pathway and activate the anti-inflammatory Nrf-2/HO-1 signal pathway. The disordered transport of Aβ across the BBB mediated by low-density lipoprotein-related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE) is a risk factor of AD pathogenesis. Formononetin treatment in APP/PS1 mice can promote LRP1-dependent Aβ clearance and inhibit the activation of the RAGE/NF-κB signaling pathway, thereby reducing the inflammatory response. However, its capability to cross the BBB is poor. (Mingzhenlong Deng et.al, 2023). Formononetin modulates many targets and in-

teracts with enzymes and receptors, altering cellular processes and molecular cascades. puerarin interactions with these targets are mapped using network pharmacology. Identifies important proteins and pathways involved in therapeutic activities [20].

Several research have used network pharmacology to examine the impact of puerarin on various diseases and biological systems. Network study shows that puerarin interacts with targets related to inflammation and oxidative damage. Potential applications include treating neurodegenerative diseases. Network pharmacology can help identify synergistic interactions between puerarin and other medicines. The network can uncover synergistic combinations of formononetin and conventional drugs to improve therapeutic efficacy and reduce side effects [13].

Experimental Material and Method

Chemical constituent: the chemical constituent was download from pubchemdatabase. the 3D structure of constituent download in the .sdf format.(https://pubchem.ncbi.nlm.nih.gov/).

Open Babel

the chemical constituent download from pubchem in .sdf format are converted into the pdbqt format with the help of open babel tool. The primary purpose of Open Babel, a computer program and biochemical skilled system, is to transform various chemical data formats.

Text mining

The various targets for every active constituent were identified with the help of databases like Swiss Target Prediction and Binding DB. Some of the other targets were also identified with the help of Literature review. Both of these database's help predict the targets based on structural features of the compounds and experimental data on the binding properties of the compounds to various proteins respectively. The canonical SMILES of the phytoconstituents was given as input in these databases to get their respective targets. The therapeutic targets of AD were identified with the help of DisGeNET database which helps in providing a thorough and evidence-based perspective of the association between diseases and the genes, and help in finding potential targets and repurpose existing drugs for different uses.

Common targets were identified with the help of Venny 2.1.0 online tool which helps producing and analysing Venn diagrams which are important for visualizing the connections between various datasets. The common targets were identified by first adding the targets of thephytochemicals used and then by adding the targets of AD and the Venn diagram created included targets of the phytochemicals with the same target as Alzheimer's Disease.

GO and KEGG pathway enrichment analyses

Gene ontology (GO) ideas and KEGG networks are frequently employed in computational biology to characterize the intricate and distinct functions occurring in mammalian cells. A well-known comprehensive database tool for gene and protein annotation is KEGG (Kyot Encyclopedia of Genes and Genomes). GO keywords and KEGG pathways can offer a more precise and transparent view of the underpinning biological mechanisms. Three characteristics of biological domains-cellular elements, molecular functions, and biological processes are covered by this paradigm in terms of our current understanding of them. Annotation network nodes are descriptions of genes or proteins. Inserted the genes obtained the text mining stage into the input set, then used the GO biological process groups to assess this gene set. The biological processes that have the greatest enrichment were chosen. The following stage involved using the genes with the chosen annotations for a second Gene Codis analysis using pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG). These pathways are substantially enriched above the P-value limit.

Protein protein interaction network

The common targets that were identified with the Venny 2.1.0 tool were added to the STRING Database and a PPI network was created by entering Homo sapiens as species. This database helps in understanding how different proteins interact with one another and offers a thorough and complete overview on PPIs and functional relationships. The topological analysis was then done with the help of Cytoscape software which helps in visualizing and analysing various biological networks. The CytoNCA plugin of cytoscape was used to assess the topological parameters like Degree Centrality (DC) and Betweenness Centrality (BC) of the network created and exported with the help of STRING database and the top proteins were identified on the basis of these topological parameters.

Cytoscape

For the massive incorporation of molecular association network data, Cytoscape is an open-source software platform with varied applications. The interaction network can be visualized and analyzed using the Cytoscape software platform. A software program called Cytoscape allows users to visually explore biological networks made up of genes, proteins, and other sorts of interactions. The tool is backed by a variety of annotations along with experimental data [27].

Molecular docking

Molecular docking is a technique that examines how molecules align and conform- collectively known as their "pose" into the binding site of a macromolecular target. This method entails forecasting the atomic-level interactions between a tiny chemical and a protein. the molecular docking was performed by the Auto dock tool. we chose the app PyRx (https://sourceforge.net/projects/pyrx/).PyRx is a open source programme for drug discovery , virtual screening, high performance, enhanced accuracy. need to add the chemical constituent that are obtained from the open babel software. and add the target genes that are obtain from the Protein data bank (PDB) in the PDB format.

Result

chemical constituent

The chemical constituent puerarin was entered in the Pubchem and obtained the 3D structure of puerarin.

Open Babel

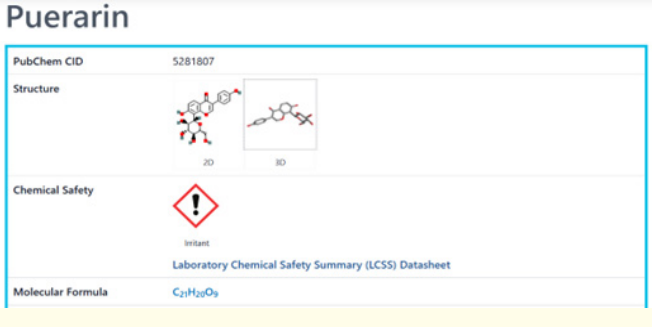

Figure 1: Pubchem structure.

The puerarin was convert from. sdfto. pdbqt format.

Text mining

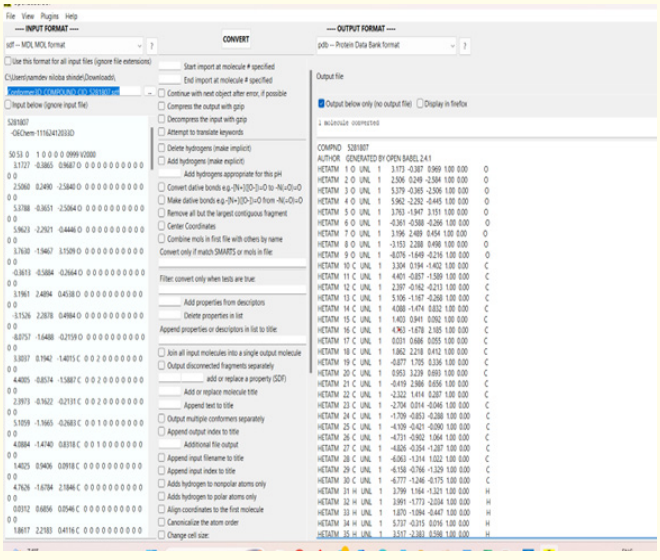

Figure 2

A total of 104 targets of the phytochemicals were identified by entering the canonical SMILES by giving input in the databases like Swiss Target Prediction and Binding DB.

Absorption, distribution , metabolism ,and excretion properties of active constituents of puerarin.

10 Top ranked target obtained from PPI network topological

Constituent	Drug likeness	BBB	Caco2	HIA	PPB
Puerarin	-0.02	1.75	6.03338	54.3978	54.3833

Table a

analysis performed using cytoscape sowtware tool.

Protein	Degree	Betweenness
TNF	55	1105.138309
AKT1	54	883.7947147
EGFR	47	687.2257451
TP53	44	570.8336869
PTGS2	42	579.021143
BCL2	42	476.7038143
PPARG	41	482.0089967
ESR1	39	526.3042531
HIF1A	36	267.191416

Table b

Venny 2.1

From the venny software the genes that are obtained from Swiss Target Prediction database and GeneCards database that are common in both database are obtain.

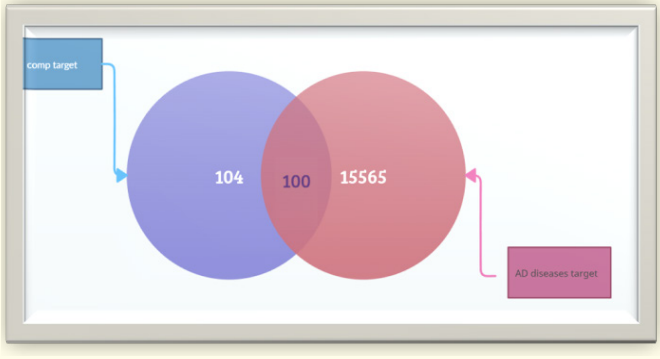

Figure 3: Venny Software Daigram of Common Targets.

GO and KEGG pathway enrichment analyses

Genecodis enrichment analysis was performed using a biological process. In the process of KEGG pathway enrichment analysis, the P-value cutoff was set at 1.00E-07.we obtained the 10 pathways related to the Positive Regulation of Protein Kinase B Signaling, Response To Amyloid-Beta, Cellular Response To Reactive Oxygen Species, Transmembrane Receptor Protein Tyrosine Kinase Signaling Pathway, Amyloid Fibril Formation, Positive Regulation Of Synaptic Transmission, Gabaergic are considered for the next From the KEGG pathway 100 genes was considered.

Protein-protein interaction

The 100 targets were identified with the help of Venny 2.1.0 tool and were added to the STRING Database PPI network. The results are given in figure 3. The PPI network helped in understanding

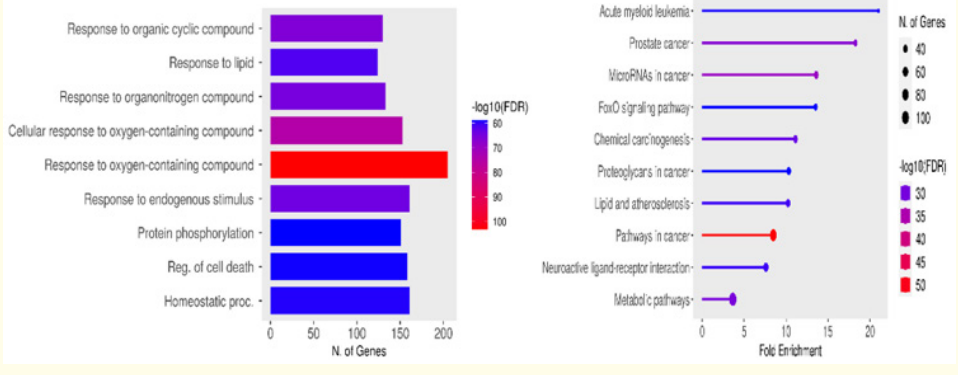

Figure 4: GO and KEGG pathway

interactions between different proteins and their functional relationships. Top 10 proteins were identified from the 100 common proteins with the help of Cystoscope software.

Cytoscape

Data was imported from string to cytoscape and degree and betweenness was applied from which genes were selected based on degree and betweenness. The more gene products that interact with one another, the higher the Degree of node, and the more significant role the gene plays in disease. The Betweenness value of the node indicates the tendency of a node to connect to the core of other nodes.

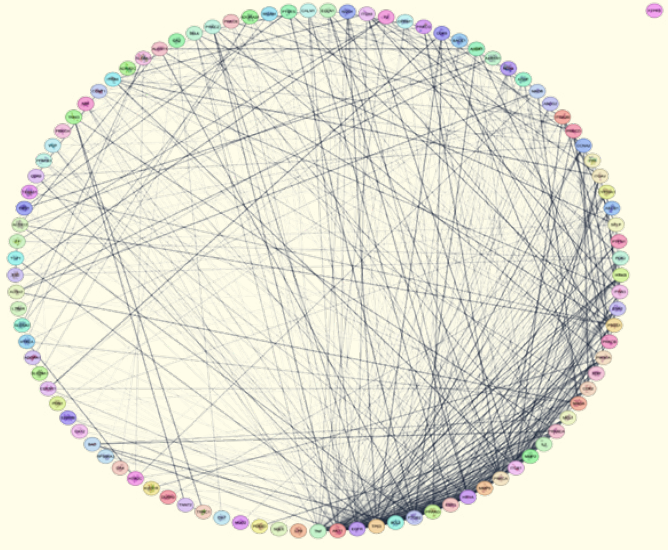

Figure 5: Protein-Protein interaction.

Molecular docking

The binding affinity of formononetin with target genes is obtained from the Autodock. It is as below.

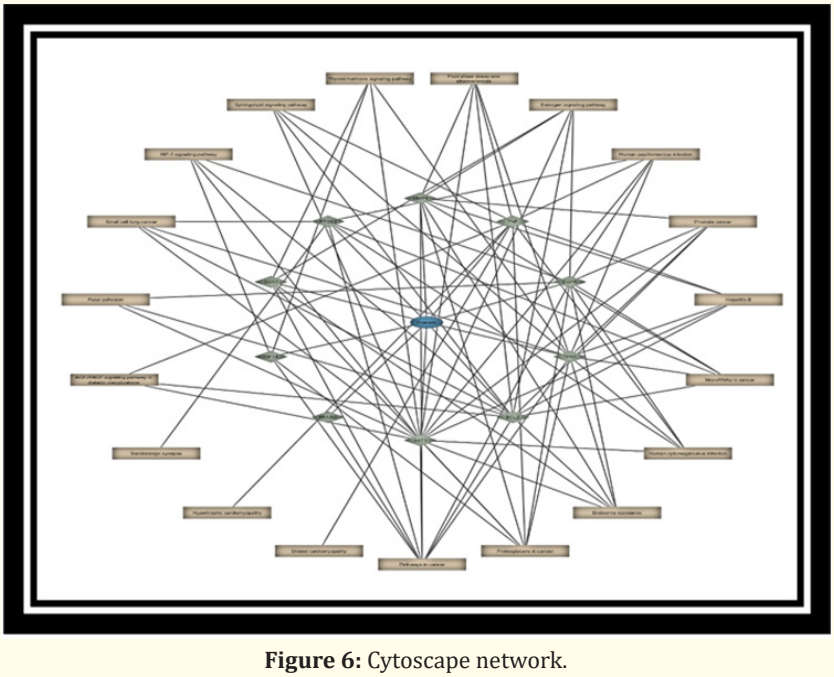

Figure 6: Cytoscape network.

Summary and Conclusion

Alzheimer's disease is a progressive neurological condition that impairs thinking and memory skills as well as daily functioning. Instead of currently available treatments, which concentrate on symptom relief, the development of novel therapies will be more advantageous for neurodegeneration. Considering novel therapies is important wherein came the concept of Network Pharmacology. It is an interdisciplinary field that combines network science, pharmacology, and systems biology to investigate the intricate relationships that exist between medications and biological systems. It aims to comprehend the ways in which biological networks are affected by pharmaceuticals and how these networks react to these perturbations. It has become a vital technique in the search for new drugs and can be applied to discover brand-new therapeutic targets, forecast drug effectiveness and toxicity, and enhance drug combinations. Network pharmacology, as opposed to classical pharmacology, focuses on the network level interactions between drugs and biological systems to provide a complete knowledge of action of drug. The goal of network pharmacology research is to understand the complex web of molecular interactions that underlie Alzheimer's disease. Researchers may build complete networks of genes, proteins, and pathways that contribute to Alzheimer's disease by combining enormous amounts of data from diverse sources, such as genomics, proteomics, and metabolomics. The identification of important nodes and pathways that can be the focus of therapeutic action is made possible by these networks, which offer a comprehensive perspective of the disease mechanisms. The discovery of therapeutic targets is one use of network pharmacology in Alzheimer's. Researchers can pinpoint proteins that are crucial to the progression of the disease by examining the protein-protein interaction networks linked to alzheimer's (Gai and Zhang et al.,2022). To control their activity and restore normal brain function, these proteins can then be targeted with particular medications or medication combinations. Network pharmacology can also aid in the prediction of probable adverse effects and drug-drug interactions from anti-alzheimer drugs. Researchers can evaluate the safety profile of medications and improve treatment approaches by fusing information on drugtarget interactions and recognised adverse drug responses. The search for new medication options to treat Alzheimer's can also be aided by network pharmacology. The discovery of unknown nodes or pathways in the disease network allows researchers to suggest novel pharmacological targets that may have therapeutic promise. Small molecules or naturally occurring compounds that interact with these targets can be found via virtual screening and computational modelling techniques, which can then be used to develop new anti-alzheimer's medications (Lin et al., 2020). In the current investigation, we first established the pharmacokinetic characteristics and toxicity of puerarin using Vinny software, and we got a total of 104 targets of puerarin against alzheimer targets. According to the degree, the top 100 targets in the puerarin - Alzheimer's PPI network were examined, and they are AKT1, TNF, EGFR, and TP53. The number one objective in the list was APP. Additionally, according to some accounts, Alzheimer's disease is brought on by the oxidative alteration of APP caused by ROS, which is viewed as the loss of activity dependent protein translation that is essential for maintaining and preserving synaptic plasticity. According to the pharmacological literature on Alzheimer's linked networks, APP is essential for Alzheimer. In alzheimer's, AKT1, TNF, and EGFR are frequently employed as proinflammatory indicators. All of the remaining core targets are crucial players in the Alzheimer's disease (Qu et al., 2021).

In conclusion, network pharmacology methods can shed light on the probable mechanisms of action of formononetin in neurological diseases like Alzheimer's and related ones. These methods can aid in the identification of important nodes, pathways, and targets linked to Alzheimer's disease by combining large-scale data and studying protein-protein interaction networks. Researchers have been looking at the possible anti-alzheimer activity of Puerarin, which have demonstrated positive neuroprotective benefits. Identification of the molecular targets through which these flavonoids affect Alzheimer's can be aided by network pharmacology. Researchers can pinpoint important proteins and signaling pathways involved in the disease process by studying the protein-protein interaction networks linked to alzheimer's. Network pharmacology can also make it easier to investigate possible therapeutic combinations that include puerarin and traditional anti-alzheimer drugs.

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