RESEARCH ARTICLE

A Network Pharmacology Approach to Explore the Potential Mechanism of *Ficus religiosa* against Alzheimer's Disease

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ABSTRACT

Background: *Ficus religiosa,* also known as the peepal tree, is used to treat multiple diseases. It is proved to be effective in treating cognitive impairment. However, the potential targets and pharmacological and molecular mechanisms of its action on the management of Alzheimer's Disease (AD) are not entirely clear. Therefore, a network pharmacology approach is required to further study and explore its treatment mechanism.

Methods: ChEBI database was used to retrieve the active constituents. AD genes were retrieved from the DisGeNet database. SMILES of phytoconstituents were retrieved from PubChem, traced for the positive drug-likeness score, targets were predicted and regulated proteins were enriched to trace probable modulated proteins, pathways, and GOterms. Cytoscape *ver*. 3.7.2 was used to construct the *active ingredient-target-pathway* interaction of *F. religiosa* and network analysis. Molecular docking was also performed.

Results: A total of 7 bioactive out of 25 were predicted to possess a positive drug likeliness score. Also, PIK3CA, PIK3RA, AKT1, MAP2K1 and RAF1 were predicted as key gene targets. The results of the GO analysis demonstrated that the somatodendritic compartment, cytoplasm, plasma membrane, and membrane raft are the maincellular components, its molecular functions are mainly catalytic activity, ion binding, protein kinase activity and, identical protein binding. The biological process is focused on biological quality, response to oxygen-contain-compound sound, and metabolic process.

Conclusions: This study holistically illuminated possible pharmacological mechanismsof *F. religiosa* that might be strongly associated with multi-targeting compounds and act on many AD pathways at the same time.

Keywords: Alzheimer's Disease, Enrichment Analysis, *Ficus religiosa*, Molecular Docking, Network Pharmacology.

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INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disorder that alters memory and judgment over time. The extracellular aggregation of amyloid peptides and intracellular inclusions of neurofibrillary tangles are pathological hallmarks of AD.[1] According to reports, the number of persons diagnosed with Alzheimer's disease globally will exceed 130 million by 2050, posing significant socioeconomic concerns.[2] The exact etiology of AD is unknown; nevertheless, it appears to include numerous pathways in the central nervous system that ends in neuronal death.[3] Current treatment in the therapy of mild to moderate dementia associated with AD loses effectiveness as the disease progresses[4] and is also linked with a set of adverse effects.[5] To counter this limitation, herbal medicine could be a preferable alternative since it contains many phytoconstituents that target multiple protein molecules, modulate various pathogenic pathways, and have a synergistic impact with few adverse effects.[6] Experimentally determining the pharmacological mechanisms of herbs with diverse Phyto constituents and targets is difficult.To understand the ligand-target interaction in diverse polygenic diseases, researchers used thetechnique of a single medication targeting many protein molecules and the lock and key model.To establish a holistic perspective and a complete and systematic insight into the processes of multi-ingredient medicine, the trend in drug development used gene set enrichment analysis and network pharmacology approach. These methodologies are being used in folk medicine todiscover the molecular mechanism of multi-component drugs to target multiple proteins and have a synergistic therapeutic effect.[7]

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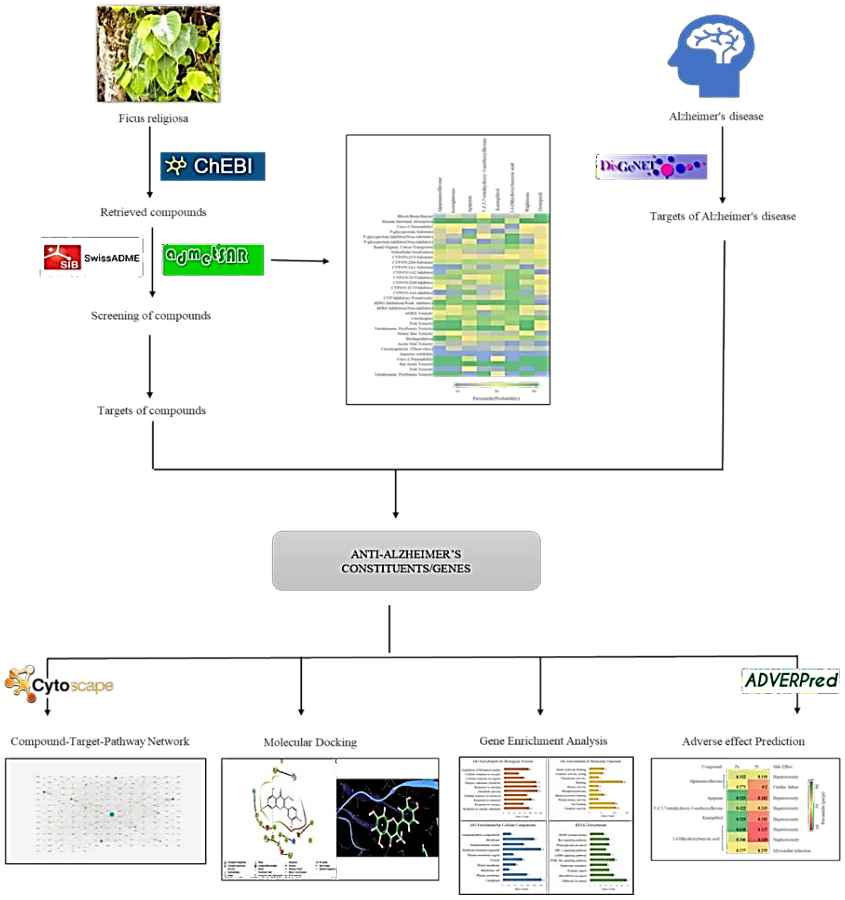


Figure 1: Schematic diagram of network pharmacology to explore the potential mechanism of *F. religiosa* for management of AD

Ficus religiosa is the most popular member of the *Moraceae* family. In Indian culture, it has mythological, religious, and medical significance. Numerous ancient holy books,including Arthasastra, Upanishads, Mahabharata, Bhagavad-Gita, and Buddhist literature, mention *F. religiosa*.[8] The nutritional composition of the plant is substantial in all parts of the tree. The fruits are high in both macro and micronutrients. Its fruits are a good source of carbohydrates, protein, and fat.[9,10] It is extensively used in alternative systems of medicine like Ayurveda, Unani, and Siddha in various formulations. It has been widely used in traditional medicine for a variety of disorders associated with the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system, and infectious disorders.[11]

MATERIALS AND METHODS

Screening of Bioactive and Target Identification from *F. religiosa*

The bioactive present in *Ficus religiosa* were retrieved from ChEBI (https://www.ebi.ac.uk/chebi/)[12]; their canonical SMILES were retrieved from PubChem (https://pubch em.ncbi.nlm.nih.gov/)[13] along with their structure (Figure 1). The targets of each phytoconstituent were retrieved from the Swiss target prediction database. All targets of each phytoconstituent involved in AD were identified concerning the DisGeNet database.[14]

Drug-likeness Prediction and ADMET Profile

Phytoconstituents were predicted for the drug-likeness score using MolSoft (http://www.molsoft.com/) and the "Lipinski's rule of five" model. Similarly, the ADMET profile of individual phytoconstituents was predicted using admetSAR2 (http://lmmd.ecust.edu.cn/admetsar2/).[15]

Prediction of Side Effects

By querying the SMILES of each phytoconstituent, ADVERpred (http://www.way2drug.com/adverpred/).[16] was used to predict the probable adverse effects. Side effects were considered if the probability of activity (Pa) was larger than the probability of inactivity (Pi) and the Pa value was more than 0.7 (Figure 2).

Gene Set Enrichment Analysis

STRING (https://string-db.org/)[17] was used to predict protein-protein interactions among the targets involved in the pathogenesis of AD. The KEGG pathway was used to identify the genes involved in the specific pathway as well as their pathway-pathway interactions.

Network Construction and Analysis

Cytoscape *ver* 3.7.2[18] was used to construct the network between phytoconstituents, targets, and pathways. The constructed network was analyzed using the 'Network analyzer' tool, which was used to set thenetwork as directed and interpret

Table 1: MF-molecular formula; MW-molecular weight; NHBA-number of hydrogen bond acceptors; N HBD-number of hydrogen bond donors'; DL Score-drug-likeness score

Compound	*MF*	*MW*	*NHBA*	*NHBD*	*Mol LogP*	*DL Score*
Alpinumisoflavone	$C_{20}H_{16}O_5$	336.1	5	2	3.66	0.23
Isowighteone	$C_{20}H_{18}O_5$	338.12	5	3	3.93	0.67
Apigenin	$C_{15}H_{10}O_5$	270.05	5	3	3.22	0.39
3',4',5,7-tetrahydroxy-3-methoxyflavone	$C_{16}H_{12}O_7$	316.06	7	4	1.92	0.53
Kaempferol	$C_{15}H_{10}O_6$	286.05	6	4	1.61	0.5
3,4-Dihydroxybenzoic acid	$C_7H_6O_4$	154.03	4	3	1.05	0.23
Wighteone	$C_{20}H_{18}O_5$	338.12	5	3	4.14	1.06

the entire network using edge count. The counts of nodes of compounds, targets, and pathways could differ based on the relied information and time of data retrieval. Different styles of nodes were used to represent phytoconstituents, targets, and pathways.

Docking Studies

The primary objective of molecular docking is to predict the ligand and receptor complex. Maestro software[19] was used to perform molecular docking analyses such as LigPrep, Prep Wizard, Grid generation, and docking calculations. Firstly, the protein for the docking investigation was chosen from the PDB databank (PDB ID- 4L23). The canonical SMILES of the constituents were mined from PubChem. The Glide SP (standardprecision) module was used to prepare and calculate molecular docking in Schrödinger Maestrosoftware.

RESULTS

Screening of Bioactive from *F. religiosa*

Twenty-five different phytoconstituents were identified in *Ficus religiosa* from ChEBI among which seven phytoconstituents were selected after ADMET analysis and positive drug-likeness scores. PubChem was used to retrieve the chemical structure of these unique compounds (Table 1). These phytoconstituents were identified as flavonoids, phenols, and isoflavone.

ADMET Profile and Drug-likeness Prediction

Seven phytoconstituents showed positive drug-likeness scores. Among them wighteone received the highest drug-likeness model score (Table 2). The ADMET profile of each phytoconstituent wasdone and predicted for their probability of human intestinal absorption, p-glycoproteinsubstrate, blood-brain barrier, carcinogens, fish toxicity acute oral toxicity, etc. The ADMET profile of each phytoconstituent is represented as a heat map (Figure 3).

Prediction of Side Effects

The probable side effects of all seven phytoconstituents were predicted. Five phytoconstituents showed probable side effects except for isowighteone and wighteone. Myocardial infarction, hepatotoxicity, nephrotoxicity, heart failure, and arrhythmia were the probable side effect of the predicted compounds (Figure 2).

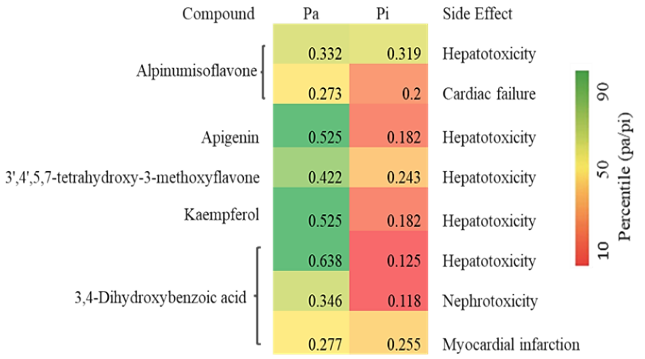


Figure 2: Probable side effects of phytoconstituents. Pa: probable activity, Pi: probable inactivity

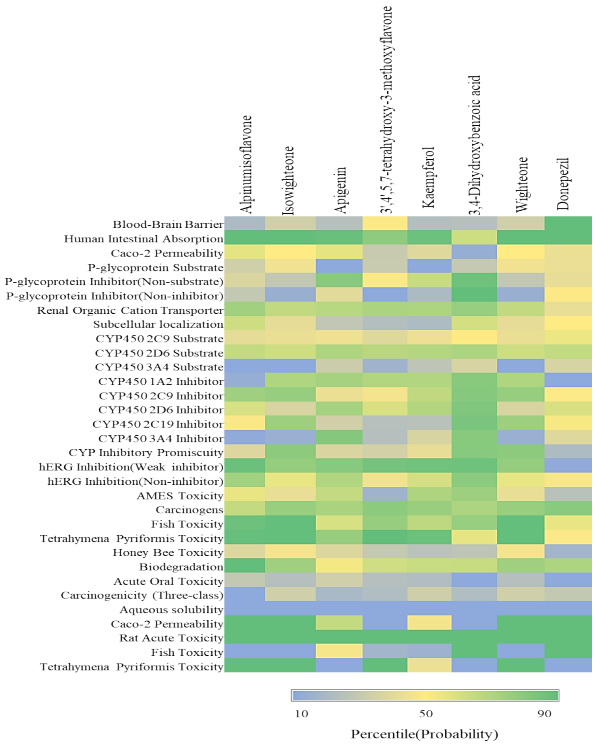
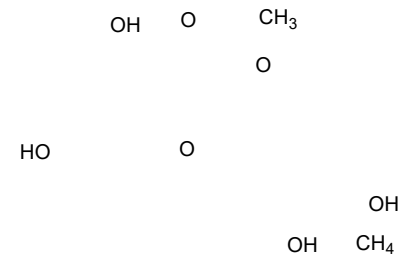
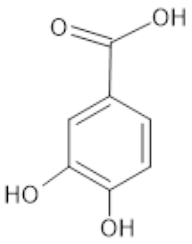
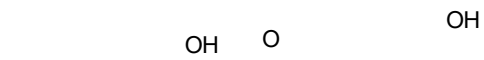
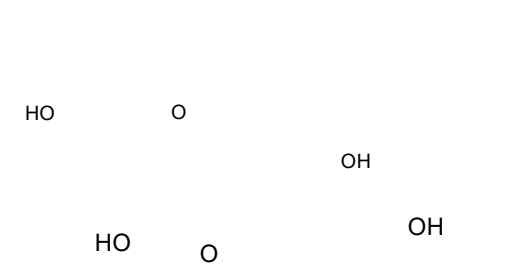
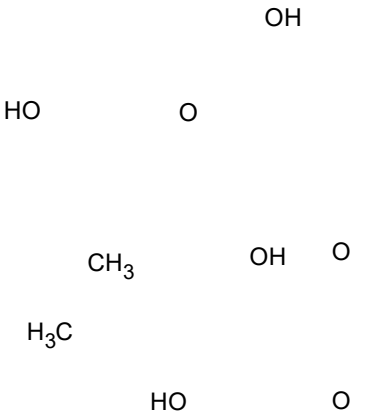


Figure 3: ADMET profile of phytoconstituents

Gene Set Enrichment Analysis

F. religiosa genes were analyzed using GO enrichment and KEGG pathway analysis. GO analysis identified 1378 biological

Table 2: Structure and class of active ingredients of *F. religiosa*

Compound	*Class of compound*	*Structure*
3',4',5,7-tetrahydroxy-3- methoxyflavone	Flavonoids	
3,4-Dihydroxybenzoic acid	Phenols	
Alpinumisoflavone	Flavonoids	
Apigenin	Flavonoids	
Isowighteone	Isoflavone	
Kaempferol	Flavonoids	
Wighteone	Flavonoids	

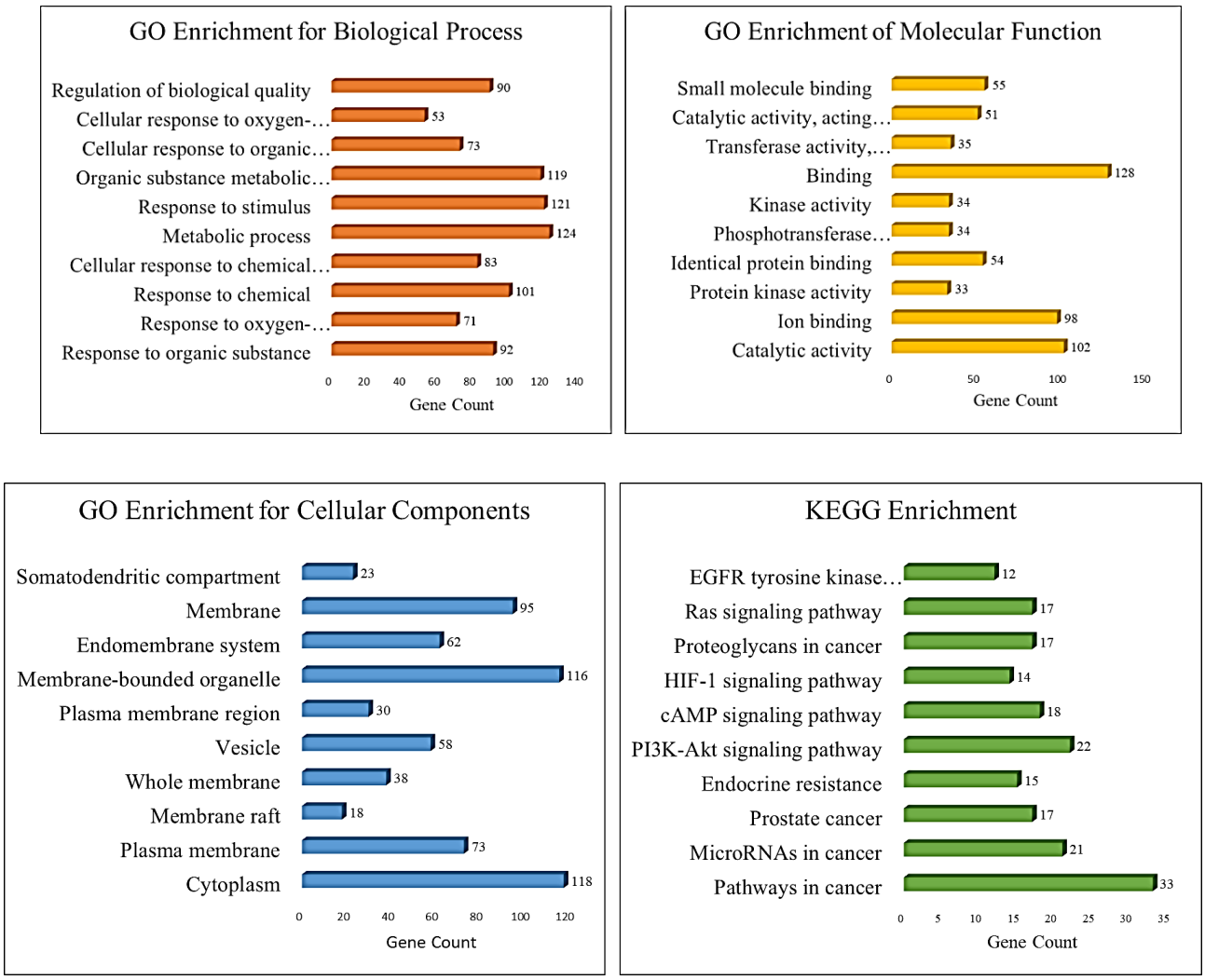


Figure 4: Gene set enrichment analysis of proteins modulated by phytoconstituents from *F. religiosa.*

Table 3: Docking results show the binding energies of potential target with compounds.

Compound	*PIK3CA*
3',4',5,7-tetrahydroxy-3-methoxyflavone	-11.003
Kaempferol	-9.508
Isowighteone	-9.238
Apigenin	-9.11
Wighteone	-8.943
Alpinumisoflavone	-8.828
3,4-Dihydroxybenzoic acid	-7.509
Donepezil	-5.839

processes (BP), such as regulation of biological quality, response to oxygen-containing compounds, metabolic process, and 89 cellular components (CC), such as the somatodendritic compartment, cytoplasm, plasma membrane, membrane raft; and 145 molecular functions (MF) such as catalytic activity ion binding, protein kinase activity, identical protein binding. Top 10 GO enrichment (BP, CC, and MF) and 10 KEGG pathways were chosen usingthe p 0.05 cut-off value. The KEGG pathway and GO enrichment were depicted (Figure 4).

Network Construction and Analysis

The network has 287 nodes and 1588 edges, with seven active components. In the "*plants- compounds-targets-pathways*" network, the alpinumisoflavone compound was expressed most towards various targets and pathways with a 45 edge count, and PIK3CA was most expressed towards various compounds and pathways with a 99 edge count (Figure 5). Pathway in cancer (*hsa05200)* was expressed the most and it was identified to regulate 33 genes against 517 background genes at 0.97 strength and 1.20E-19 false discover rate. The targets of *F. religiosa* phytoconstituents show coordination with diverse paths and are connected to each other, and play a crucial role in the management of AD.

Molecular Docking

All seven phytoconstituents from *F. religiosa* and donepezil as a standard drug were docked with the target. A docking study was performed to check the affinity of phytoconstituents with

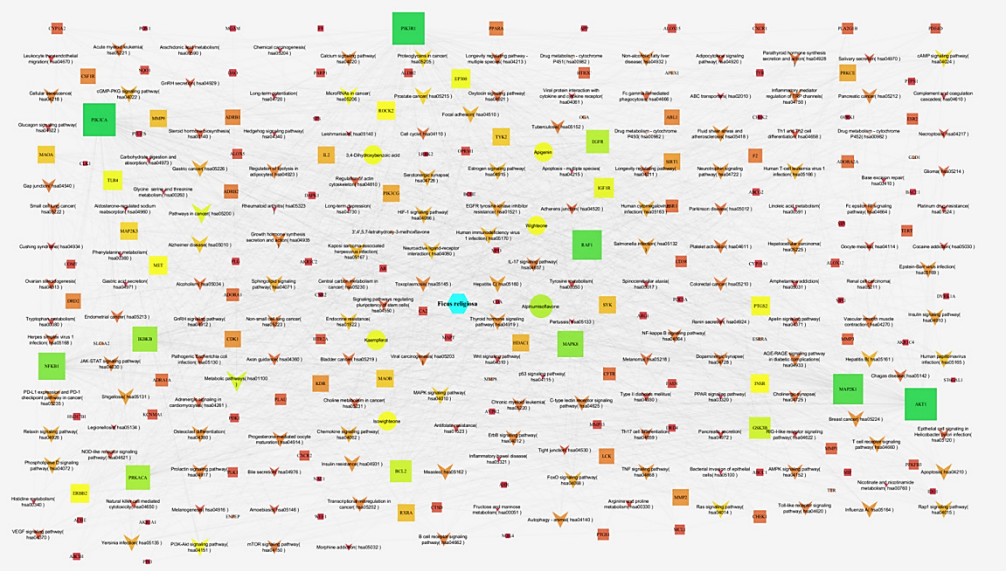


Figure 5: "Plants-compounds-targets-pathways" network. Round shape represents phytoconstituents, diamond shape represents protein targets, V/down-arrow represents pathways and the hexagon represent the plant.

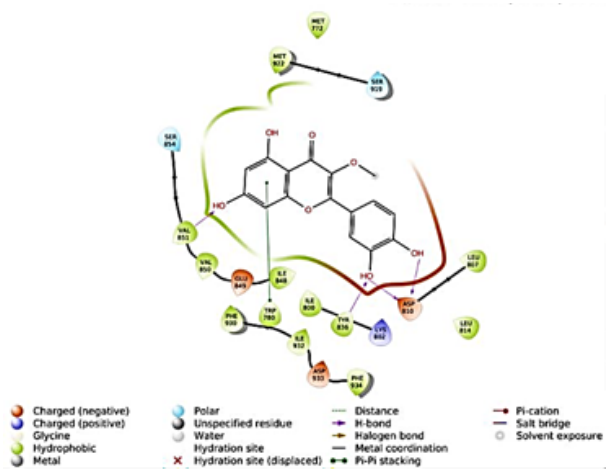


Figure 6: 2D plot of interaction of3',4',5,7-tetrahydroxy-3-methoxyflavone with PIK3CA.

PIK3CA (PDB ID- 4L23) protein molecules. Docking study revealed that 3',4',5,7-tetrahydroxy-3-methoxyflavone, and Kaempferol scored the highest affinity with PIK3CA *i.e.,* -11.003 and -9.508 kcal/mol, respectively (Figure 6). Docking scores of interactions between all phytoconstituents and target is depicted in Table 3.

DISCUSSION

Network pharmacology approaches facilitate better understanding of the numerous connections and associations that exist between medicines, their targets, and their probable mechanisms of action.[20-22]

AD is a complex neurodegenerative disorder marked by a progressive loss of cognitive function triggered by several etiological factors whose etiology and pathophysiology remain unknown.[23] As a result, rather than monotherapy, combination treatments are required for the production of effective approaches to treating AD.[24] Aducanumab, Donepezil, Rivastigmine, Galantamine, etc. are the drugs available for the management of AD with several side effects.[25] As a result, the search for effective medication for the management of AD with lesser side effects has become more focused. A rich source of phytoconstituents with health benefits could be a suitable drug candidate for the management of AD.

F. religiosa, known as the peepal tree or sacred fig tree, is a tree that possesses numeroushealth benefits like Anti-diabetic, Anti-inflammatory, Anti-ulcer activity, Wound healing, Anti-asthmatic, Anti-Parkinson, and many more.[26] *F. religiosa* is rich in phenols, tannins, steroids, alkaloids, flavonoids, etc.[27] It is reported to have memory-enhancing property dueto presence of amino acids, sterols, glycosides, and tannins.[28] The current study emphasized elucidating the underlying mechanism of *F. religiosa* for the management of AD by utilizing gene set enrichment analysis, network pharmacology, and molecular docking study.

The identified phytoconstituents which followed "Lipinski's rule" with positive drug likeliness properties were utilized to know the possible protein target concerning the Swiss target prediction database. The proteins involved in AD were retrieved from the DisGeNet database. Among numerous gene targets PIK3CA, PIK3R1, AKT1, MAP2K1, and RAF1, etc. were the highly expressed genes. According to KEGG pathway analysis, multiple gene targets of *F. religiosa* played an important role in numerous AD-related pathways. Genes like BACE1, MAPT, NOX4, APP PIK3CA, and the other 10 target genes are involved in Alzheimer's pathway which is the main targeted pathway in this study. BACE1 (β-site APP cleaving enzyme 1 or -secretase) is the focus of AD research

because it is implicatedin the abnormal development of amyloid plaques (Aβ), which is the indication of its pathophysiology. Evidence suggests that there is a strong link between AD and BACE1. BACE1 appears to be a prime target for preventing Aβ generation in AD due to its apparent rate-limiting function.[29] PIK3CA and PIK3R1 are the genes that command the making of p110 alpha (p110α) protein, which is the subunit of an enzyme phosphatidylinositol 3-kinase (PI3K) which is involved in PI3K-AKT signaling pathway.[30]

When investigating the association between T2D and AD risk, the insulin PI3K-AKT signalling pathway has been found to prevent excessive accumulation of Aβ protein and abnormal phosphorylation of tau protein that contributed to senile plaques and neurofibrillary tangles in AD by downregulating GSK3 level.[31] As a result, the insulin PI3K-AKT signaling pathway is thought to represent the pathobiochemical basis for Alzheimer's brain's dramatic glucose/energy metabolism decline.[32] The MAP2K1 gene encodes a protein known as MEK1 protein kinase.[33] The abnormal hyperphosphorylation of tau in AD has been proposed to involve the extracellular-signal-regulated protein kinase (ERK) of the mitogen-activated protein kinase (MAPK) family. ERK is phosphorylated and thereby activated by MAP kinase (MEK). Hence, targeting the MAP2K1 gene can be helpful in the management of AD.[34]

By network analysis, PI3KCA was taken as the target for docking studies to check the binding affinity with seven active compounds of *F. religiosa* as anti-Alzheimer's activity. 3',4',5,7-tetrahydroxy-3-methoxyflavone was successfully docked with PI3KCA with high binding affinity compared to other compounds. Flavonoids were reported to play an important in the management of AD. Further network analysis reveals that *F. religiosa* has anti-disease Alzheimer's effects by reducing the prime targets of the disease. The current study elaborates on the active chemicals, their potential targets, and linked pathways for the treatment of AD in terms of network pharmacology, providing a conceptual foundation for additional experimental exploration.

CONCLUSION

This work sheds new scientific insights on the effectiveness of multi-component, multi-target drug regimens and identifies more AD therapy targets. The molecular mechanism *of F. religiosa* for treating AD was determined using network pharmacology and a molecular docking technique. Pathway in cancer, metabolic pathway, PI3K-Akt signaling pathway, cAMP signaling pathway, MAPK signaling pathway, etc., was identified as prime pathways regulated by these potentially bioactive phytoconstituents; this supports a potential involvement in the management of AD. According to network analysis, *F. religiosa* has multi-targeting compounds that act on many AD pathways at the same time. Furthermore, results revealed that the PIK3CA, PIK3RA, AKT1, MAP2KI, and RAF1 genes are viable and effective therapeutic targets for preventing and managing Alzheimer's disease, potentially resulting in positive efficacy. Nonetheless, the current study has certain limitations, as more phytochemical and pharmacological research is required to substantiate our findings.

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