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Network pharmacology and molecular docking-based insights into the anti-glomerulonephritis potential of *Calendula officinalis*

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Abstract: Glomerulonephritis (GN) is a kidney disorder characterized by inflammation of the glomeruli. This study aims to explore the potential therapeutic mechanisms of *C. officinalis* in the treatment of GN through network pharmacology and molecular docking analysis. The active ingredients of the plant were obtained from the intersections of articles and databases. The GN targets were obtained from the GeneCards database. The STRING database was used to construct a PPI network obtained from the intersection of target and disease. Functional enrichment analysis was performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) to examine the biological significance of disease and plant active ingredient targets. Quercetin, isorhamnetin and kaempferol were identified as key compounds, while TNF, AKT1 and SRC emerged as central targets in the PPI network. GO and KEGG analyses revealed that *C. officinalis* may exert its effects through processes on important signaling pathways including PI3K-Akt and EGFR tyrosine kinase inhibitor resistance. Molecular docking results provided important affinities between the main compounds and the core proteins. This study provides a preliminary scientific foundation for future investigations into the molecular mechanisms by which *C. officinalis* may contribute to the treatment of glomerulonephritis.

Keywords: *Calendula officinalis*; Ethnopharmacology; Glomerulonephritis; Molecular docking; Network pharmacology

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INTRODUCTION

The kidneys are critically important organs that fulfill essential physiological functions, including the filtration of metabolic waste products, the maintenance of electrolyte balance and the regulation of the body's fluid equilibrium (Madsen *et al*., 2000). The microscopic building blocks that filter blood through the kidneys and initiate the first stage of urine formation are called glomeruli (Hughson *et al*., 2003). When these microscopic building blocks become inflamed for different reasons, an inflammatory disease called glomerulonephritis occurs (Hricik *et al*., 1998).

Glomerulonephritis (GN) is a serious disease caused by inflammation of the glomerular structures of the kidneys leading to proteinuria, hematuria and impaired renal function (Chadban *et al*., 2005). In addition, GN reduces the filtering capacity of the kidneys and can cause very serious kidney damage if left untreated (Han *et al*., 2020). The acute form of glomerulonephritis, which is classified into acute and chronic forms, may develop after an infectious agent and may lead to glomerular inflammation after overactivation of the immune system. In contrast, chronic glomerulonephritis is usually associated with autoimmune mechanisms and may progress more slowly, leading to long-term loss of renal function (Becherucci *et al*., 2016; Kellum *et al*., 2018). The causes of GN, which is defined as a multifactorial disease, include genetic predisposition, autoimmune diseases, infections, immunological effects and peripheral factors from the environment (Huang *et al*., 2023; Satoskar *et al*., 2020). Autoimmune diseases and immune-mediated processes are among the major causes of GN (Sethi *et al*., 2022; O'Brien, 2023). It has also been documented that environmental toxins and some drugs may lead to inflammation in glomerular structures (Xu *et al*., 2018; National Kidney Foundation, 2024). Depending on the underlying cause and severity of GN, treatment of the acute form is usually symptomatic, while chronic glomerulonephritis is treated with immunosuppressive drugs and angiotensin-converting enzyme inhibitors (Jones *et al*., 2010; O'Brien, 2023; Walsh *et al*., 2020).

The term ethnopharmacology was coined in 1967 and is now recognized as a field concerned with the study of traditional medicines, usually derived from plants (Houghton, 2009). For the treatment of many diseases, people prefer the therapy effects of bioactive drugs derived from medicinal plants as a better and safer alternative to synthetic drugs (Cotton, 1996; Baytop, 1999; Kendir *et al*., 2010; Yesilada, 2013; Karimi *et al*., 2017).

Calendula officinalis, which belongs to the Asteracae family, has medicinal use in many countries like America, Turkiye, China, India and Europe (Zournatzis *et al*., 2025). When we look at the traditional use of the plant, it is seen that it is used in the treatment of diseases such as peptic and duodenal ulcers, gastrointestinal system disorders,

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dysmenorrhoea in women and inflammation of organs such as the spleen and liver with its anti-inflammatory, analgesic and antiseptic effect (Chakraborthy, 2010; Singh *et al*., 2011; Albulescu *et al*., 2015). In a review article, the Asteraceae family was identified as the most frequently cited family for the treatment of kidney diseases, with 300 taxa distributed in 70 families (Kultur *et al*., 2021). The *C. officinalis* plant to be used in our study belongs to this family and has been investigated by computational approaches for the treatment of GN disease.

Network Pharmacology is a broad discipline that studies disease mechanisms and drug mechanisms of action via systems biology, pharmaceutical chemistry, pharmacology and biochemistry (Li *et al*., 2023). The main objective of network pharmacology is to explore the mechanisms of action of drugs on diseases by creating biological networks of the interactions of active drug molecules with diseases at multiple levels and systematically in complex disease mechanisms (Chen *et al*., 2018; Hopkins, 2008; Song *et al*., 2018; Zhang *et al*., 2019).

From the 1970s onward, molecular docking has played a significant role in various scientific domains, particularly in computational drug discovery and the analysis of protein–protein interactions. As a computer-based modeling technique, it evaluates the compatibility and configuration of small compounds within the binding sites of molecular targets (Wodak *et al*., 1978; Torres *et al*., 2019). This method calculates the binding energy and predicts the binding stability by evaluating the congruence of 3D structures between ligand and receptor to predict how a drug candidate binds with the target protein and how strong this binding is (Meng *et al*., 2011; Pinzi *et al*., 2019). Bioinformatics approaches help to analyze with different strategies depending on the type of disease (cancer, genetic, infectious diseases, etc.) to be evaluated for drug discovery (Driel *et al*., 2006). Bioinformatics analyses are used to process and interpret large biological data sets such as genetics, proteomics and metabolomics to determine therapeutic efficacy by understanding the molecular basis of diseases, to support methods such as network pharmacology and molecular docking (Noor *et al*., 2022).

In this study, we planned to gain a new perspective with network pharmacology and molecular docking technology by collecting information about the active ingredients' targets in *C. officinalis* and GN targets from various databases to obtain the potency of *C. officinalis* in therapeutic process of GN. The flowchart of our study is given in fig. 1.

MATERIALS AND METHODS

Active ingredients and targets of Calendulla officinalis

All active compounds of *C. officinalis* were compiled from the data obtained from the articles published by Ashwlayan *et al*. and Ak *et al*., as well as from TCMSP, IMPPAT, Dr. Duke's Phytochemical and Ethnobotanical Databases (Ak *et al*. 2020; Ashwlayan *et al*. 2018). TCMSP is a systems pharmacology database used for drug discovery (Ru *et al*., 2014). IMPPAT is a database of Indian medicinal plants (Mohanraj *et al*., 2018). Dr Duke's Phytochemical and Ethnobotanical Database is a data source that provides extensive information plants and their ethnobotanical uses (Duke, 1992). The amount of drug entering the circulatory system is called oral bioavailability. Drug similarity (DL) is expressed as the similarity between a chemical of the drug and a known drug and oral bioavailability (OB) and drug similarity (DL) data of the determined effective compounds were obtained from TCMSP (Ru *et al*., 2014). The active components of *C. officinalis* were evaluated according to absorption, distribution, metabolism and excretion (ADME) criteria and pharmacokinetic parameters were determined in accordance with the literature and TCMSP recommendations. In this context, OB $\geq$ 30% and DL $\geq$ 0.18 values were taken as selection criteria (Yue *et al*., 2017; Zhu *et al*., 2018).

Predicted targets of glomeruloneprithis

GN disease targets were obtained by searching GeneCards database the keyword 'glomerulonephritis' (Supplemantary 1) (Stelzer *et al*., 2016). All sections within the GeneCards database (comprising 18 distinct data categories) and all GeneCards gene categories (totaling 7 categories) were subjected to comprehensive and meticulous filtering. No filtering criteria were applied to the relevance scores, thereby ensuring the preservation of the full biological significance of the interactions and preventing the exclusion of potentially important associations.

Determination of active compound-target network

The target genes of *C. officinalis* were obtained from the SwissTargetPrediction database. (Supplemantary 2) (Daina *et al*., 2019). Cytoscape is a network biology visualisation and analysis application (Shannon *et al*., 2003). For visualisation, a network of GN and targets were created, with potential active constituents of *C. officinalis* and their matching targets imported into Cytoscape 3.10.3. In a compound-target network, each component or target is represented by a node and the relationship between the component and the target is indicated by a connecting line (Shannon *et al*., 2003; Otasek *et al*., 2019).

Determination of protein- protein ınteraction network

To identify intersections of active compound in the plant and targets overlapping with GN, GN targets of *C. officinalis* were imported into the jvenn database. Jvenn is an open source JavaScript library designed for use on web-based platforms for creating Venn diagrams. (Bardou *et al*., 2014). The intersecting genes were transferred to the STRING database (Szklarczyk *et al*., 2023). The drug-disease intersection genes were transferred to the STRING

database and the species was identified as '*Homo sapiens'*. Cytoscape 3.10.3 can calculate parameters such as degree, betweenness centrality (BC), closeness centrality (CC) of each node in the network diagram (Gan *et al*., 2021; Liu *et al*., 2021). Protein-protein interaction (PPI) data were retrieved based on interactions of medium confidence level or higher (score ≥ 0.400) and target nodes with BC and CC degree values were selected and 10 possible core targets were determined. In our study, a threshold value of ≥0.4- corresponding to a medium confidence level-was selected to ensure the preservation of the biological significance of the interactions and to avoid the exclusion of potentially important associations.

GO enrichment and KEGG pathway analysis

Metascape v3.5, is an efficient and useful tool for experimental biologists in the era of big data, enabling comprehensive analysis and interpretation of OMICs-based studies. All genes in the genome were used and all elements were screened according to the criteria of p value <0.01 and minimum 3 counts (Zhou *et al*., 2019). SRPLOT, a data visualisation and graphing platform, was used for visualisation. SRplot is a fast, accessible and comprehensive graphing tool for biomedical researchers (Tang *et al*., 2023).

Docking studies

For the docking study, firstly, after the target protein was determined, the 3D structure of the protein was taken from the PDB data bank. PDB is a data repository containing three-dimensional (3D) structures of biological macromolecules (Burley *et al*., 2021). 3D molecular structures of the ligands were downloaded from PubChem database (Kim *et al*., 2019). Molecular docking studies were investigated using the Maestro Schodinger package programme (Schrodinger Release 2022-4: Maestro *et al*., 2022). The molecular docking study was first started with the protein preparation step and then ligand preparation and docking was performed using the OPLS4 method. (Schrodinger Release 2022-4: LigPrep *et al*., 2022; Schrodinger Release 2022-4: Protein Preparation Wizard; Epik *et al*., 2022).

RESULTS

Active ingredients of Calendula officinalis

Active ingredients obtained from databases and review articles were screened in TCMSP according to OB> 30% and DL> 0.18. in table 1. The results obtained are as follows: Terpenoid derivative 6 components (beta-sitosterol – stigmasterol – campesterol – isofucosterol – faradiol – cholesterol) Flavonoid derivative 3 components (quercetin – isorhamnetin – kaempferol) Carotenoid derivative 3 components (alpha-carotene – rubixanthin – lycopene) a total of 12 active components were identified (Table 1) (Kaya *et al*., 2023).

Analysis of compound-target network

In the compound-target network of *C. officinalis* as shown in fig. 2, there are 125 nodes and 613 edges. All genes were represented using gene symbols determined by the HUGO Gene Nomenclature Committee (HGNC) to avoid incompatibilities between databases and platforms (Jin *et al*., 2021; Li *et al*., 2021; Wu *et al*., 2020; Yang *et al*., 2020).

Analysis and determination of PPI network

GN disease targets defined by the disease database were combined after removal of duplicate data to obtain disease targets. There are 110 common targets between the targets according to the Venn diagram results (Fig. 3). After uploading the protein-protein interaction (PPI) network, drug-disease intersection genes and STRING database, 110 nodes and 1221 edges were created in the network (Fig. 4). We further analyzed the core targets using the Maximum Clinical Centrality (MCC) algorithm in the CytoHubba plugin of Cytoscape 3.10.3. The first 10 proteins were identified as TNF, AKT1, SRC, EGFR, PPARG, PTGS2, BCL2, MMP9, ESR1 and HIF1A according to DC (Degree Centrality), BC (Betweenness Centrality) and CC (Closeness Centrality) degrees as shown in table 2 (Fig. 5).

Analysis of KEGG pathway and GO enrichment

The 110 intersection targets obtained were mapped to the Metascape database. The first 30 items were selected from the terms obtained by GO analysis. As shown in fig. 6, we screened 10 biological function, cell function and molecular function items in GO analysis, such as peptidyl tyrosine phosphorylation and modification as well as positive regulation in lipid metabolic processes and cellular response to chemical stress. We performed KEGG enrichment analysis to explore the signalling pathway mechanism of *C. officinalis* in GN disease treatment. As shown in fig. 7, it shows the top 10 signalling pathways including PI3K-Akt signalling pathway, EGFR tyrosine kinase inhibitor resistance and so on.

Docking results

According to the molecular docking results given in table 3, quercetin, isorhamnetin and kaempferol are the best compounds among the 12 active compounds in the plant content. These compounds belonging to the flavonoid group have the lowest binding energy (highest binding affinity).

DISCUSSION

The aim of our study was to carry out *in slico* analyses to conduct a preliminary study on the active components and mechanisms of action of *C. officinalis* in the treatment of GN. We have compiled 12 chemical components obtained from multiple databases. Terpenoid, flavonoid and carotenoid-derived bioactive components of plant origin are of great importance in the treatment of many diseases (Rubin *et al*., 2017; Kim *et al*., 2020; Singh *et al*., 2022).

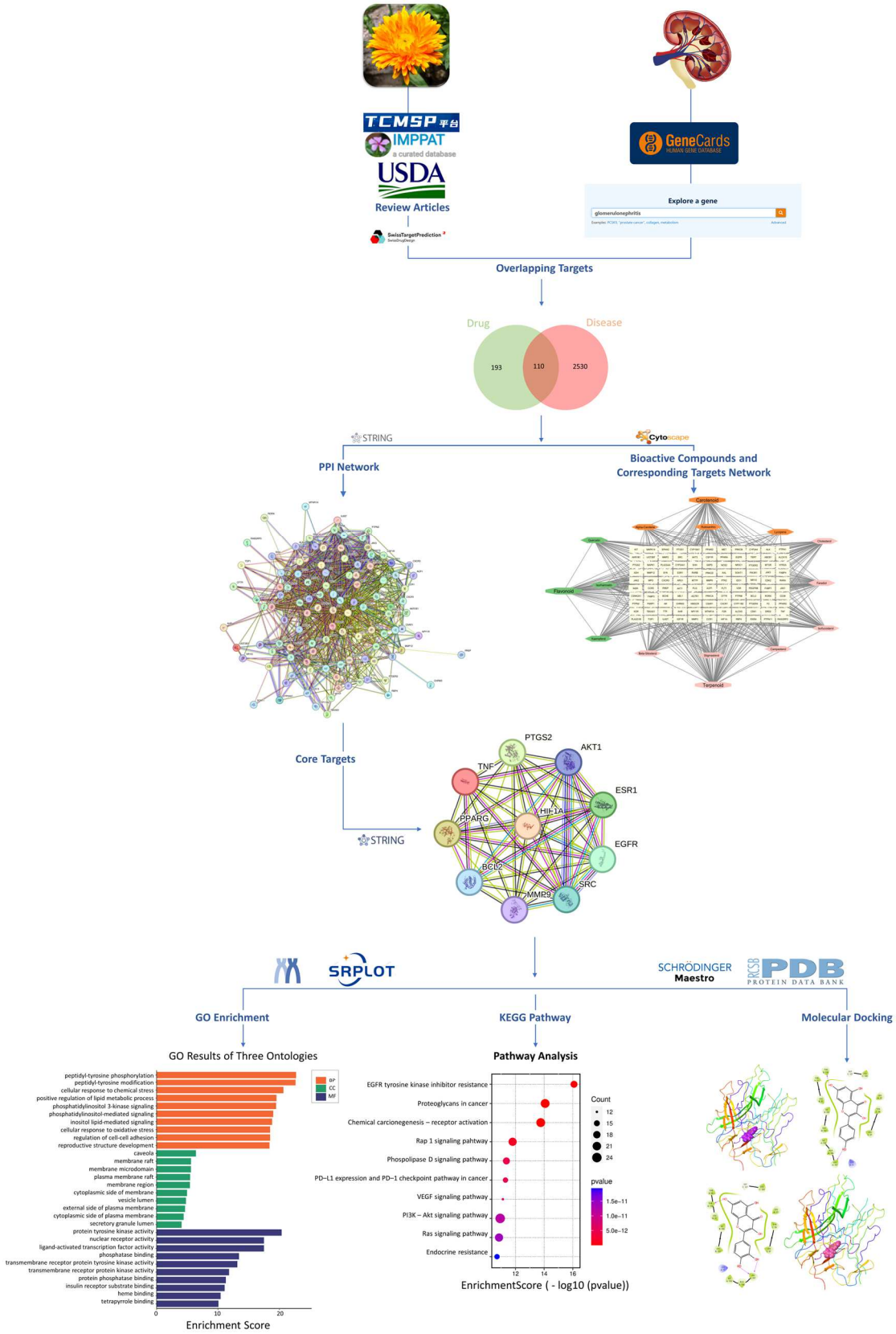


Fig. 1: The whole framework of network pharmacological study of *C. officinalis* on GN.

Table 1: Potential active compounds of *C. officinalis* (Kaya *et al*., 2023).

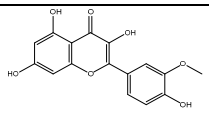
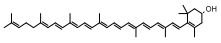
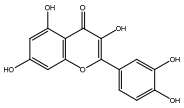
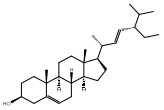
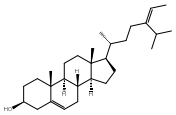
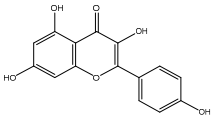
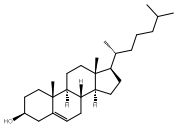
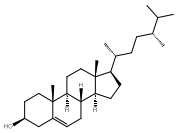
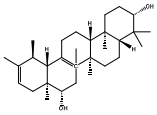
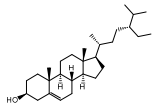
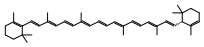
Molecule ID	Molecule structure	Compound	OB (%)	DL
MOL000354		Isorhamnetin	49.60	0.31
MOL010736		Rubixanthin	47.26	0.53
MOL000098		Quercetin	46.43	0.28
MOL002045		Stigmasterol	43.41	0.76
MOL005440		Isofucosterol	43.78	0.76
MOL000422		Kaempferol	41.88	0.24
MOL000953		Cholesterol	37.87	0.68
MOL012254		Campesterol	37.58	0.71
MOL006098		Faradiol	37.47	0.75
MOL000359		Beta-Sitosterol	36.91	0.75
MOL008691		Alpha-Carotene	34.51	0.58
MOL010267		Lycopene	32.57	0.51

Table 2: Information on attributes of the top 10 target according to the degree value.

Name	Degree centrality	Betweenness centrality	Closeness centrality
TNF	76.0	0.402291070	1.0
AKT1	71.0	1.127582300	0.772357723
SRC	64.0	0.298531452	0.714285714
EGFR	63.0	1.597399548	0.768292682
PPARG	59.0	1.174997574	0.676470588
PTGS2	58.0	0.460629071	0.75
BCL2	55.0	0.758626693	0.654135338
MMP9	54.0	0.758465860	0.679245283
ESR1	53.0	1.395200968	0.709302325
HIF1A	52.0	1.158360875	0.698795180

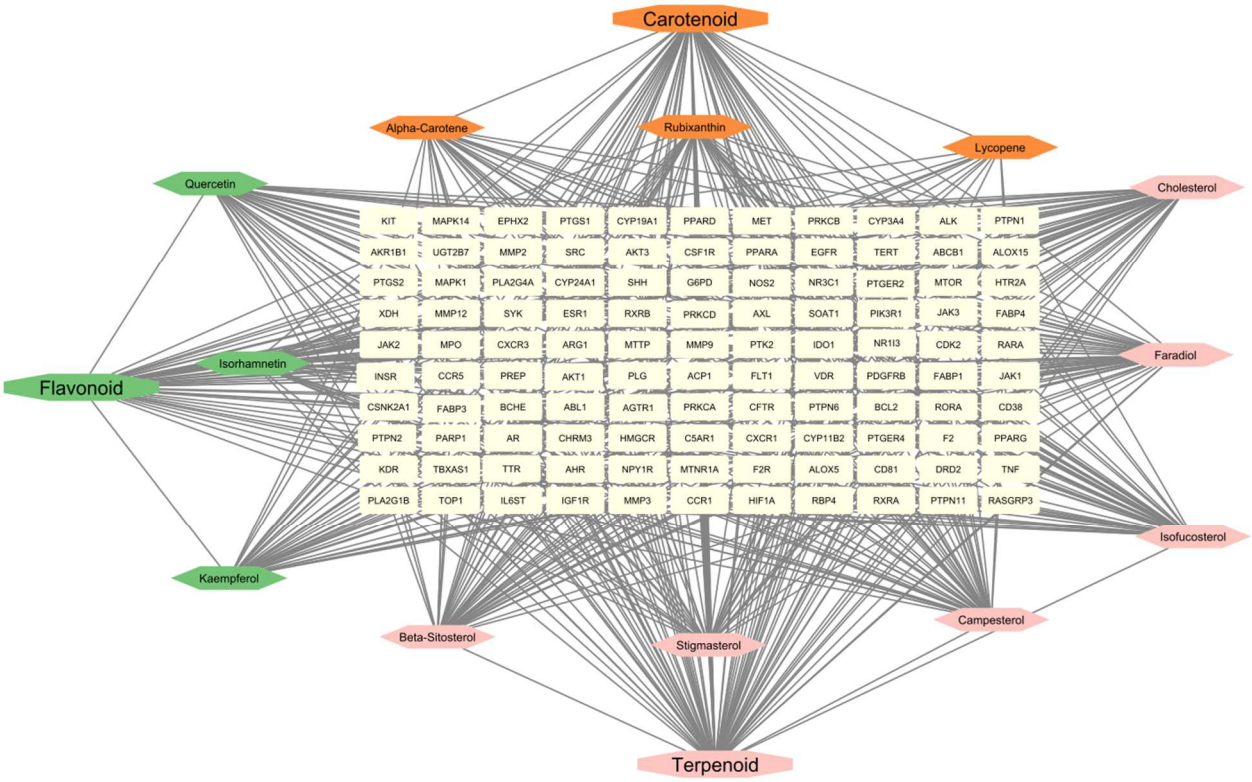


Fig. 2: Bioactive compounds and corresponding targets network of *C. officinalis* of glomerulonephritis. Octagons represent Phytochemistry of types. 3 green hexagon shapes represent flavonoid class compounds. 3 orange Hexagon shapes represent caretenoid class compounds. 6 pink hexagon shapes represent terpenoid class compounds. Yellow rectangles represent 110 potential target genes of *C. officinalis.*

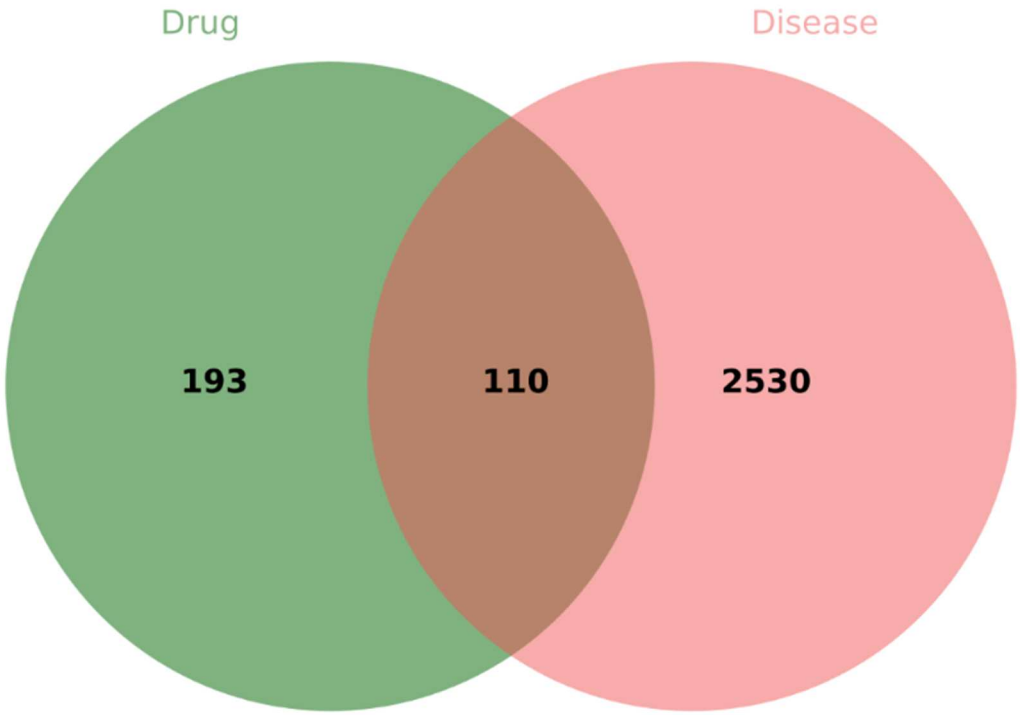


Fig. 3: Venn diagram shows genes between GN and *C. officinalis*.

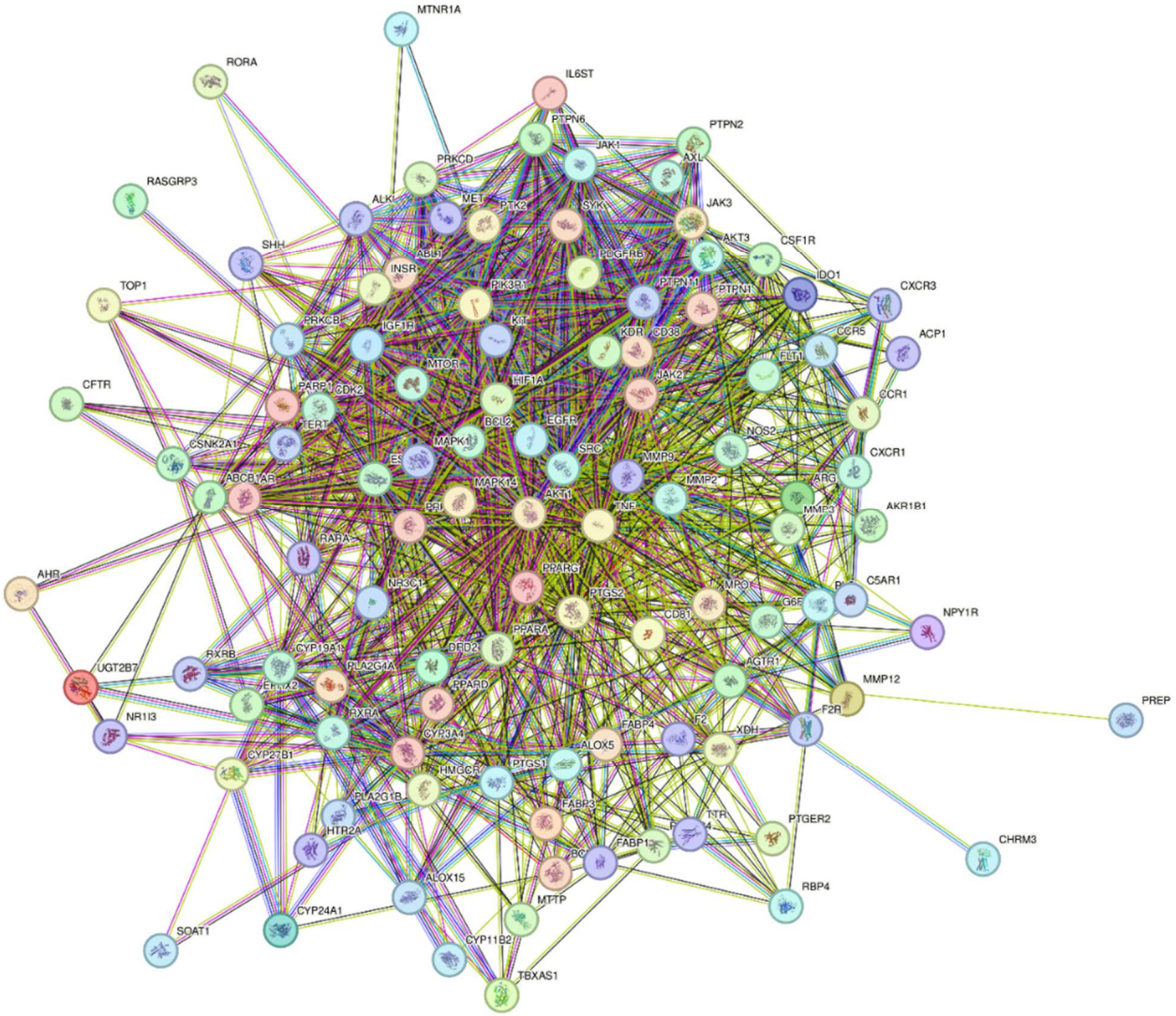


Fig. 4: PPI network between GN and *C. officinalis*. All 110 intersection targets network.

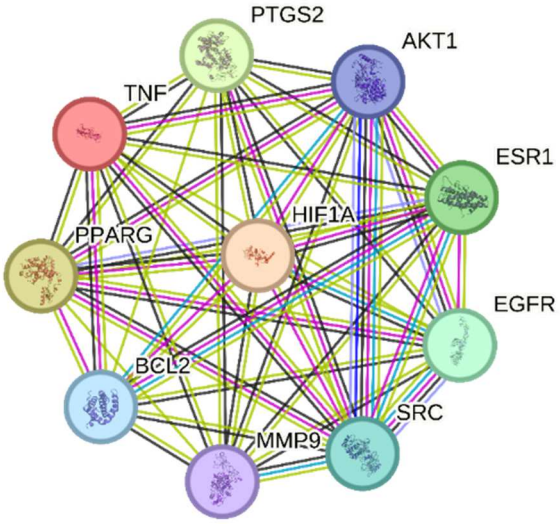


Fig. 5: Top 10 of 110 intersection targets network.

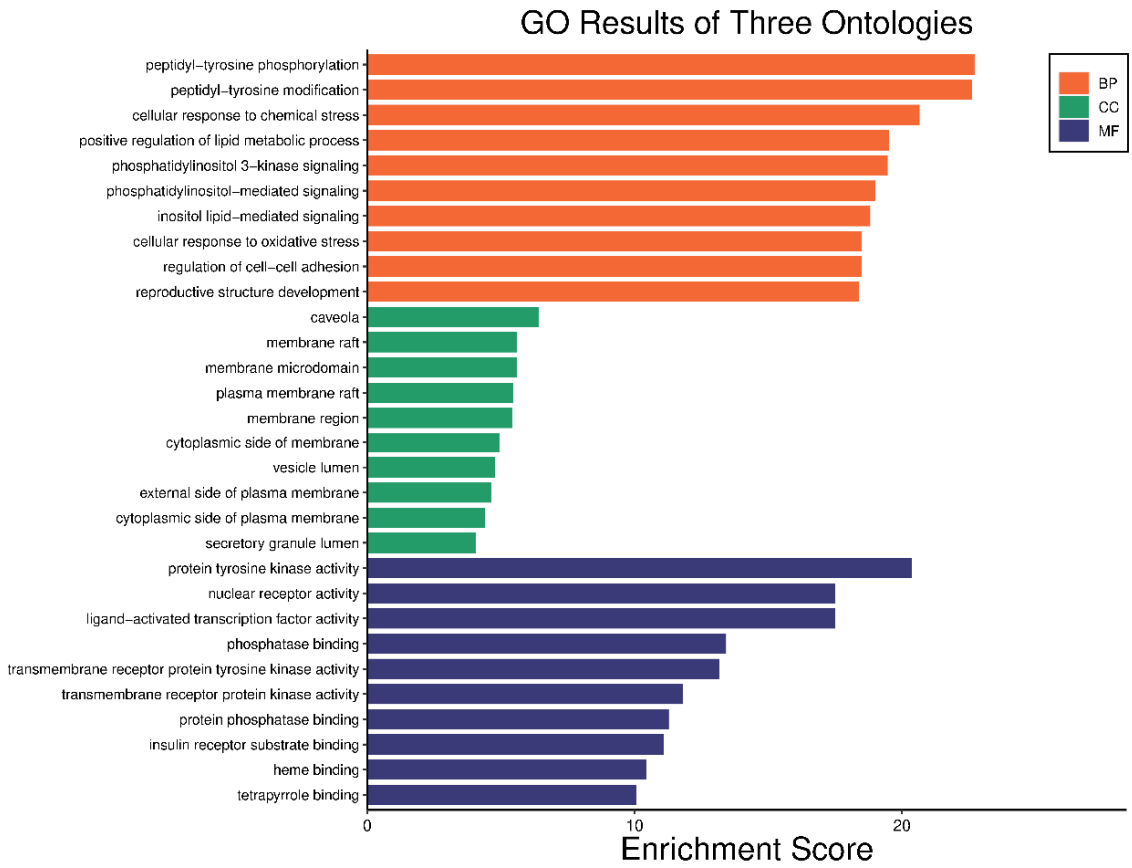


Fig. 6: Top 10 GO terms of hub genes.

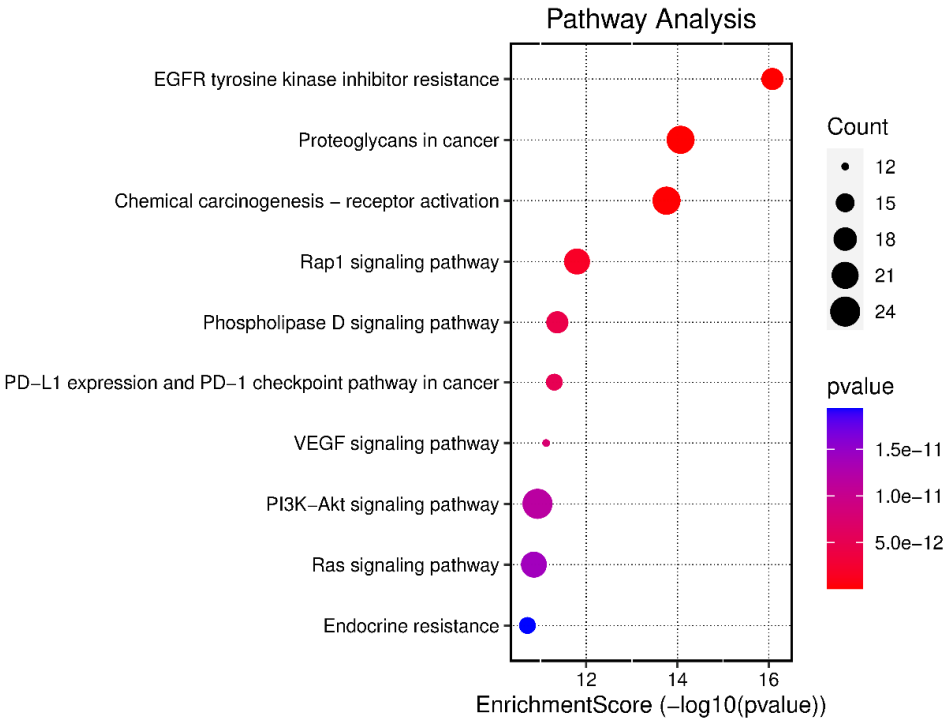
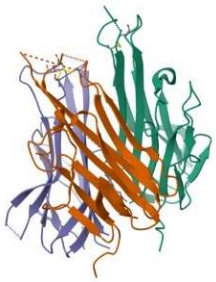
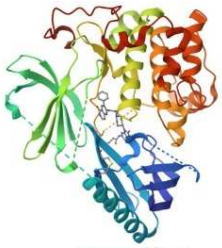
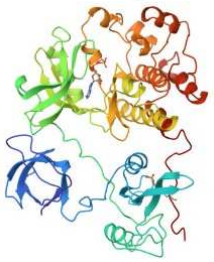


Fig. 7: Top 10 KEGG pathway of hub genes.

Table 3: Molecular docking results

Target	Target name	PDB ID	Structure	Compound	Docking score
TNF	Tumor necrosis factor	6OP0		Quercetin Isorhamnetin Kaempferol Prednisolone	-8.658 -8.229 -8.758 -8.162
AKT1	AKT Serine/threonine kinase 1	6HHF		Quercetin Isorhamnetin Kaempferol Prednisolone	-7.658 -8.369 -7.850 -6.881
SRC	Proto-oncogene tyrosine-protein kinase	2SRC		Quercetin Isorhamnetin Kaempferol Prednisolone	-7.719 -7.733 -7.702 -6.084

The common importance of these compounds are their role in the treatment of inflammation-related diseases by regulating inflammatory responses and preventing cellular damage by helping to reduce oxidative stress (García-Lafuente *et al*., 2009; Supriyadi et al., 2023).

The protective effects of quercetin, a flavonoid compound, were investigated in a study of 5-FU (5-fluorouracil)-induced nephrotoxicity in rats. At the end of the study, it was determined that 5-FU caused an increase in kidney function tests such as urea, creatinine and BUN levels and that quercetin, administered for protective purposes, was particularly effective at a dose of 100 mg/kg (Kansu *et al*., 2022). Diabetic nephropathy can lead to chronic renal failure in patients. D-ribose triggers mitochondrial apoptosis by inducing AGE (Advanced Glycation End Products) aggregation and ROS (Reactive Oxygen Species) production in diabetic patients and mouse models, but kaempferol may have a protective effect (Zhang *et al*., 2019). Qiu *et al*. (2017) demonstrated that isorhamnetin may have a protective effect on the kidneys through negative regulation of the NF-κB signaling pathway and reduction of oxidative stress in ischemia-induced acute kidney injury (Qiu *et al*., 2017).

In this study, a PPI network was constructed from 110 intersection genes expressing protein-protein interactions to discover key targets. The results of the PPI network revealed that TNF, AKT1, SRC, EGFR, PPARG, PTGS2, BCL2, MMP9, ESR1 and HIF1A especially TNF, AKT1 and SRC, could be core targets. With the STRING database, Node Degree, which shows how many connections a node has with other nodes, Betweenness Centrality, which measures how much a node controls the information flow in the network and Closeness Centrality, which expresses how close a node is to other nodes in the network, were obtained. These data show that the network structure is strongly interconnected and that it is possible to reveal the intensity of interactions between proteins and the potential for these interactions to contribute to important biological functions. In fig. 5, there are 45 edges between the 10 target nodes. Among the edges, light blue indicates information from recorded and validated databases, pink indicates protein interactions determined by experimental studies, green indicates relationships between genes in the same genome region, red indicates protein-protein interactions formed by the combination of different genes, dark blue indicates interactions of genes observed simultaneously in different species, yellow indicates protein interactions determined by text mining, black indicates relationships based on the co-expression level of proteins, purple indicates interactions based on homologous (similarity) relationships of proteins (Li *et al*., 2022; Szklarczyk *et al*., 2023).

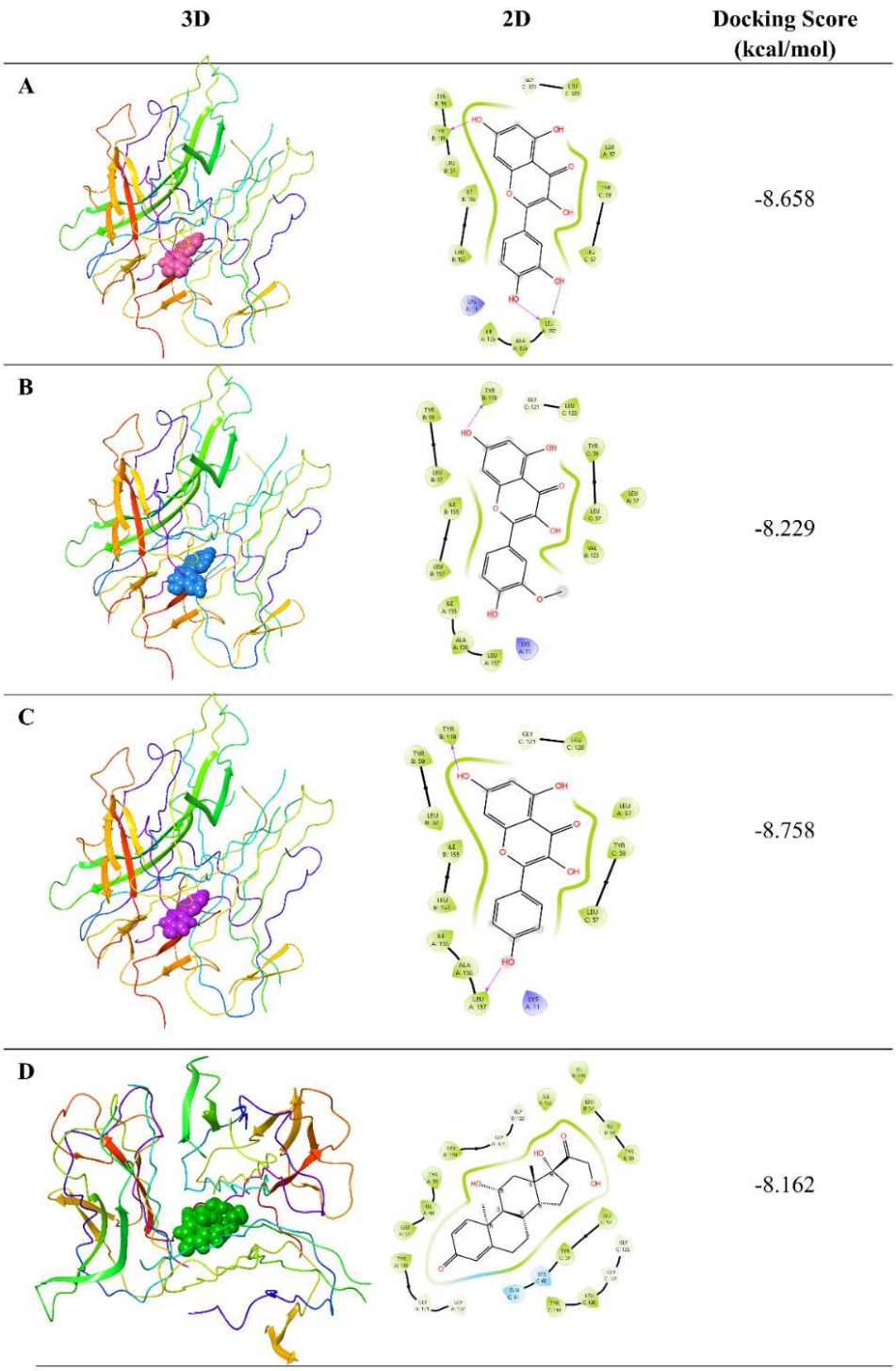


Fig. 8: Molecular docking 3D, 2D models and docking score of TNF with bioactive compounds. (A) TNF – Quercetin (B) TNF – Isorhamnetin (C) TNF – Kaempferol (D) TNF – Prednisolone

In the TNF–quercetin interaction, two hydrogen bonds were formed with LEU157 and one hydrogen bond with TYR119. The docking score was determined as −8.658 kcal/mol. In the TNF–isorhamnetin interaction, one hydrogen bond was formed with TYR119, with a docking score of −8.229 kcal/mol. In the TNF–kaempferol interaction, one hydrogen bond was formed with TYR119 and one with LEU157, and the docking score was determined as −8.758 kcal/mol. In the TNF–prednisolone interaction, the "Ligand Interaction" module did not automatically activate hydrogen bond visualization for certain poses, and the bonds did not meet specific distance/angle criteria. As a result, hydrophobic interactions and van der Waals bonds were visualized, and the docking score was determined as −8.162 kcal/mol.

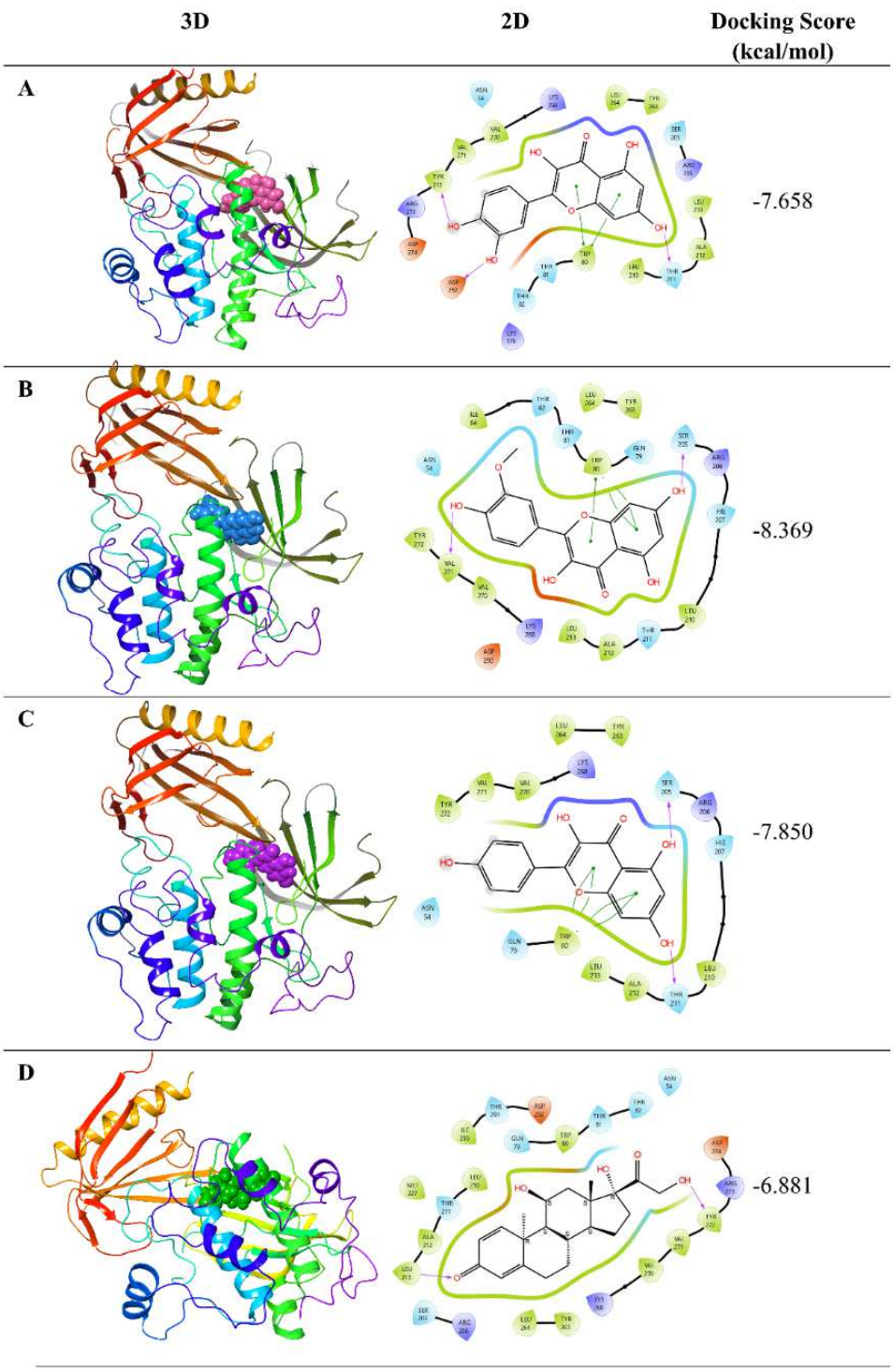


Fig. 9: Molecular docking 3D, 2D models and docking score of AKT1 with bioactive compounds. (A) AKT1 – Quercetin (B) AKT1 – Isorhamnetin (C) AKT1 – Kaempferol (D) AKT1 – Prednisolone

In the AKT1–quercetin interaction, one hydrogen bond was formed with TYR272, ASP292, and THR211, along with a π–π interaction with TRP80. The docking score was determined as −7.658 kcal/mol. This helps the molecule remain more stable within the binding region. In the AKT1–isorhamnetin interaction, one hydrogen bond was formed with VAL271 and SER205, along with a π–π interaction with TRP80. The docking score was determined as −8.369 kcal/mol. In the AKT1–kaempferol interaction, one hydrogen bond was formed with THR211 and SER205, along with a π–π interaction with TRP80, and the docking score was determined as −7.850 kcal/mol. In the AKT1–prednisolone interaction, one hydrogen bond was formed with LEU213 and TYR272. The docking score was determined as −6.881 kcal/mol.

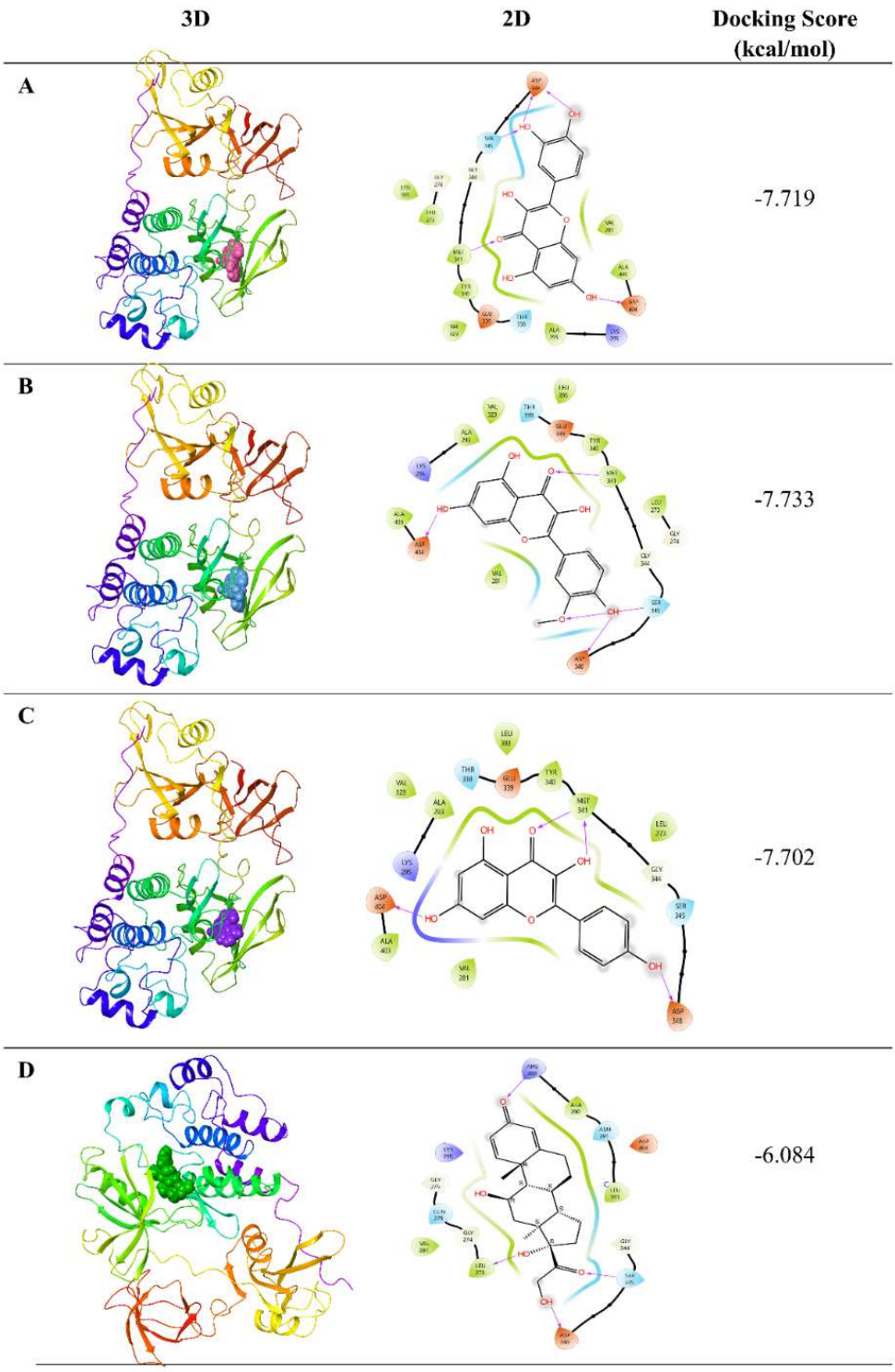


Fig. 10: Molecular docking 3D, 2D models and docking score of SRC with bioactive compounds. (A) SRC – Quercetin (B) SRC– Isorhamnetin (C) SRC – Kaempferol (D) SRC – Prednisolone

In the SRC–quercetin interaction, two hydrogen bonds were formed with ASP348, and one hydrogen bond each was formed with SER345, MET341, and ASP404. The docking score was determined as −7.719 kcal/mol. In the SRC–isorhamnetin interaction, one hydrogen bond was formed with ASP404, MET341, SER345, and ASP348. The docking score was determined as −7.733 kcal/mol. In the SRC–kaempferol interaction, two hydrogen bonds were formed with MET341, and one hydrogen bond each with ASP404 and ASP348. The docking score was determined as −7.702 kcal/mol. In the SRC–prednisolone interaction, one hydrogen bond was formed with LEU273, ASP348, SER345, and ARG388. The docking score was determined as −6.084 kcal/mol.

Most of these genes, selected according to their Degree Centrality (DC), Betweenness Centrality (BC) and Closeness Centrality (CC) features, have important effects on basic cellular activities such as signal transduction, cell proliferation, differentiation, apoptosis and inflammation. Khan *et al*. (2005), reported that neutralization of endogenous TNF-alpha was effective in reducing inflammation in the kidneys, significantly preventing fibrosis formation and preserving kidney function in the treatment of experimentally induced crescentic glomerulonephritis (Khan *et al*., 2005). In addition, certain candidate antigens retained in the glomeruli play a role in the development of glomerular inflammation by stimulating the release of IL-6, tumor necrosis factor (TNF), IL-8 and transforming growth factor-β (TGF-β) from peripheral blood leukocytes (Yoshizawa *et al*., 2004; Rodriguez-Iturbe, 2021; Anders *et al*., 2023). Kim *et al*. (2021), reported that AKT1 has an effective importance in the process of progression of kidney damage from acute to chronic in the treatment of kidney diseases (Kim *et al*., 2021). Wen *et al*. (2025) reported that, based on immunohistochemical results from rat kidney tissues divided into various groups with induced immune complex deposition and glomerular inflammation, AKT1 levels increased significantly, while the tested compound reduced AKT1 levels, suggesting its potential importance in the treatment of chronic GN (Wen *et al*., 2025). Studies by Yan *et al*. (2016) reported that Overexpression of SRC may contribute to the progression of renal tumors and certain glomerular diseases. When SRC inhibitors are administered, fibroblast activation is reduced, collagen and other matrix protein accumulation in the tissue is reduced, activation of the TGF-β1, EGFR and STAT3 signaling pathways is suppressed and the retention of cells in the G2/M phase (renal cell cycle dysfunction) is also reduced (Yan *et al*., 2016).

GO analysis and KEGG analysis were used to understand the metabolic pathways and functions of associated proteins in the treatment of GN disease of *C. officinalis*. GO analysis results show that the target genes are concentrated in biological functions such as peptidyl-tyrosine phosphorylation, peptidyl-tyrosine modification and cellular response to chemical stress. It has been emphasized that, in intracellular signal transduction, processes such as peptidyl-tyrosine phosphorylation and peptidyl-tyrosine modification, involving protein kinases such as AKT1 and SRC, may represent an important pathological mechanism in nephrotic syndrome (Wang *et al*., 2020). The results of KEGG enrichment analysis show that *C. officinalis* plant has PI3K-Akt signalling pathway, EGFR tyrosine kinase inhibitor resistance, proteoglycans and chemical carcinogenesis-receptor activation pathways. Therefore, considering that these pathways are very important in the metabolic-physiological processes of cells and are effective in the progression of many intracellular mechanisms, it is very important to show that the effective compounds of *C. officinalis* have an effect on these pathways.

EGFR is a member of the human epidermal receptor family of tyrosine kinase receptors that can pass through the cell membrane. These tyrosine kinase receptors are used in the treatment of cancers caused by the overactivation or mutation of EGFRs. These inhibitors bind competitively with ATP and reversibly inhibit the tyrosine kinase domain of EGFR. Additionally, they activate the BCL2-like 11 (BIM)-mediated apoptosis pathway, leading to the death of tumor cells (Huang *et al*., 2015). Tyrosine kinase inhibitors (TKIs) are the most commonly used targeted drugs in the treatment of clear cell renal cell carcinoma (ccRCC), but it has been reported that the use of these drugs may lead to tumour progression if care is not taken (Marona *et al*., 2022).

In many kidney diseases such as chronic glomerulonephritis, it has been reported that activation or inhibition of the PI3K/Akt signalling pathway can reduce kidney damage by regulating many processes in the body (inflammatory response, apoptosis, autophagy and epithelial-mesenchymal transformation (EMT)) (Wang *et al*., 2024). The study determined that the PI3K–Akt–mTOR signaling pathway is activated during peritubular myofibroblastic transdifferentiation (PMT). The PI3K–Akt pathway regulates numerous cellular processes, including proliferation, apoptosis, inflammation and fibrosis (Chen *et al*., 2023). It has been reported that inhibition of this pathway, which plays a key role in the pathogenesis of chronic glomerulonephritis, may reduce inflammation, fibrosis and glomerular injury (Wen *et al*., 2025). After the PPI analysis, it was seen that the best core targets were determined as TNF, AKT1 and SRC genes and the compounds with the best docking score among 12 effective compounds in the plant content were kaempferol, isorhamnetin and quercetin from the flavonoid class. The binding image and the connections it made are shown in figs. 8-10. Flavonoids, which have antihypertensive, antidiabetic and anti-inflammatory effects, show potential renoprotective effects in diseases such as glomerulonephritis, diabetic nephropathy and chemically induced renal failure (Vargas *et al*., 2018).

The kaempferol compound has the highest binding affinity for TNF; Tumor Necrosis Factor with -8.758 kcal/mol value, the second compound with the highest binding affinity is quercetin -8.658 kcal/mol and the third highest affinity is obtained from isorhamnetin -8.229 kcal/mol. In a study, sepsis-induced acute kidney injury, sepsis mouse model was created and kaempferol treatment was applied. As a result, kaempferol was shown to alleviate kidney damage (Xu *et al*., 2023).

For AKT1; AKT serine/threonine kinase 1 the highest binding affinity is exhibited by isorhamnetin -8.369

kcal/mol, followed by kaempferol -7.850 kcal/mol and quercetin -7.658 kcal/mol.

SRC; proto-oncogene non-receptor tyrosine kinase, has the highest binding affinity for isorhamnetin -7.733 kcal/mol, followed by quercetin -7.719 kcal/mol and kaempferol -7.702 kcal/mol. Our reference drug, prednisolone, exhibited the weakest binding compared to the other compounds, with docking scores of −8.162 kcal/mol, −6.881 kcal/mol and −6.084 kcal/mol against TNF, AKT1 and SRC proteins, respectively. In a study, it was reported that isorhamnetin attenuated renal fibrosis by regulating endogenous H_2S production and Keap1-Nrf2 signalling pathway (Zhang *et al*., 2024).

In this context, our docking study is very important to determine the potential effects of plant compounds, especially the flavonoid group, found in the structure of the *C. officinalis* plant on GN-related biological targets such as TNF, AKT1 and SRC. In particular, the docking study with the reference drug revealed that flavonoid-class compounds possess a notably high potential to inhibit biological targets that emerge following disease onset. Based on molecular docking analyses, binding energies below approximately -5 kcal/mol generally suggest a stronger receptor–ligand interaction and imply that the resulting complex adopts a more energetically favorable and stable conformation (Li *et al*., 2022; Liu *et al*., 2021; Zeng *et al*., 2021).

In conclusion, TNF, as a pro-inflammatory cytokine, enhances immune cell infiltration in the glomeruli and initiates pro-fibrotic processes (Taguchi *et al*., 2021). AKT1 regulates cell proliferation, apoptosis and inflammation via the PI3K/Akt signaling pathway; excessive activation of this pathway leads to fibrosis and chronic damage in glomerular cells (Wang *et al*., 2024). SRC kinase, through tyrosine phosphorylation mechanisms, activates signaling pathways such as EGFR and STAT3, thereby promoting mesangial cell proliferation and matrix accumulation (Pace *et al*., 2019). Regulation of the PI3K–Akt and EGFR-associated pathways—where these three targets converge-plays a pivotal role in controlling both inflammatory responses and structural glomerular alterations. Thus, it is anticipated that the flavonoid constituents of *C. officinalis* may exert a renoprotective effect against GN by inhibiting these pathways.

The TNF, AKT1 and SRC genes not only drive inflammation and cell proliferation in GN pathogenesis but also activate specific mechanisms of glomerular injury. The PI3K–Akt and MAPK signaling pathways emerge as key regulators of cellular injury, inflammation and fibrosis in the pathophysiology of GN. The PI3K–Akt pathway controls processes such as cell proliferation, apoptosis and autophagy, while its excessive activation promotes fibrosis and heightened immune responses in glomerular cells (Miricescu *et al*., 2021). The MAPK (ERK, JNK, p38) pathway, on the other hand, amplifies oxidative stress and the release of pro-inflammatory cytokines (TNF, IL-6), triggering mesangial cell proliferation and matrix accumulation (Jubaidi *et al*., 2022). In GN, these two pathways frequently engage in cross-talk; for instance, PI3K–Akt activation can potentiate MAPK signaling, thereby perpetuating the inflammatory cycle. This dual modulation may prevent apoptosis in glomerular cells and attenuate inflammation and fibrosis, thereby exerting a renoprotective effect against GN.

CONCLUSION

This study was conducted to reveal the basic chemical components of *C. officinalis* that may be effective in the treatment of GN and to reveal their possible mechanisms by using *in silico* calculation programmes. As a result, according to the data we obtained, it was shown that isorhamnetin, kaempferol and quercetin molecules may be the basic bioactive components of *C. officinalis* that are responsible for the main therapeutic activity in the treatment of GN. As a result of the PPI analysis performed as protein targets, it was shown that TNF, AKT1 and SRC may be targets with potential therapeutic effects in the treatment of GN. Furthermore, based on KEGG analysis results, the bioactive components we identified may be associated with TNF, AKT1 and SRC targets through the PI3K-Akt signaling pathway, EGFR pathway and cancer proteoglycan pathways. While this study is highly original in investigating the interactions of plant-derived bioactive compounds with numerous genes and pathways potentially associated with GN, the lack of *in vivo* and *in vitro* experiments presents certain limitations. For future research, conducting animal experiments with an LPS-induced nephritis model, followed by histopathological and biochemical analyses to confirm the disease model, would be highly valuable. Subsequent evaluation of gene expression levels for TNF, AKT1, SRC, EGFR, PPARG, PTGS2, BCL2, MMP9, ESR1, HIF-1α, IL-6, VEGFA and β-actin using the RT-qPCR method, along with ELISA assays to determine the cellular protein levels of these gene products, would provide strong experimental support for assessing the therapeutic potential of *C. officinalis* in GN. In addition, *in vitro* experiments such as cell culture studies and albumin denaturation inhibition assays could be performed to compare and validate the findings through applied approaches. Overall, this study serves as a highly valuable and informative reference for guiding future research.

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Authors' contributions

Seyda Kaya: Contributed to conceptualization, data curation, analysis, methodology, investigation, project administration, resource management, visualization, and the writing and revision of the manuscript.

Eda Sonmez Gurer: Provided project funding and resources; contributed to visualization and manuscript review and editing.

Sevgi Durna Dastan: Responsible for conceptualization, data curation, funding, methodology development, investigation, project administration, resource management, and supervision; contributed to drafting and revising the manuscript.

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Data availability statement

Data supporting the findings of this study are available from the corresponding author, upon reasonable request.

Ethical approval

Ethical approval is not required for this project.

Conflict of interest

The authors declare that they have no conflicts of interest related to the conduct or publication of this study.

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