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Exploring Anti-inflammatory Targets of Flavonoids through Integrated Molecular Docking and Network Pharmacology

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ABSTRACT

Inflammation is a complex physiological response associated with numerous diseases. Flavonoids, a class of natural compounds widely distributed in plants, have demonstrated promising anti-inflammatory properties. However, their comprehensive mechanisms of action and potential molecular targets remain indefinable. In the present study, we employed a network pharmacology approach combined with molecular docking to investigate the anti-inflammatory effects of some flavonoids. Initially, we collected and curated a comprehensive database such as *ADMET* parameters and targets from Swiss *ADME*, *ADMET* 2.O and *Swiss* target predication. We further constructed a protein-protein interaction network to identify key proteins involved in inflammation by using string database. Subsequently, we integrated the flavonoid dataset with the protein network to establish potential flavonoid-protein interactions by using *Cytosacpe* vina. The *GO* and *KEGG* enrichment analysis were done with the help of David database. Molecular docking was accomplished through Autodock Vina, and assessed the binding affinity of selected flavonoids towards the identified target proteins. The docking analysis provided insights into the specific interactions between flavonoids and target proteins, elucidating the potential mechanisms underlying their anti-inflammatory effects. The bioactive components ferulic acid, quercetin, rutin and hesperidin modulates many molecular and cellular processes and then exerts anti-inflammatory effects. From the analysis the key targets were participated in inflammatory bowel disease, IL 17 signaling pathway, TNF signaling pathway, cytokine-mediated signaling pathway, rheumatoid arthritis, lipopolysaccharides etc. Further molecular docking studies also revealed that binding affinity of selected flavonoids were higher than that of diclofenac.

Keywords: Anti-inflammatory; Ferulic acid; Hesperidin; Molecular docking; Network Pharmacology; Quercetin; Rutin.

INTRODUCTION

Globally, it is acknowledged that inflammatory illnesses constitute a substantial contributor to morbidity in humans [1]. Inflammation is a fundamental biological response of the immune system to tissue injury, infection, or other harmful stimuli [2]. It is a complex process involving various immune and non-immune cells, chemicals, and molecular signaling pathways that work together to protect the body and initiate the healing process [3]. There are various types of inflammation, while acute inflammation is an essential part of the body's defense mechanism, it is characterized by redness, heat, swelling, discomfort, and functional loss [1] and it is a vital part of the immune response and plays a crucial role in initiating the healing process. Whereas chronic inflammation can lead to tissue damage and contribute to the development of

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numerous diseases [4] such as multiple sclerosis, psoriasis, inflammatory bowel syndrome, autoimmune disorder (rheumatoid arthritis), cardiovascular diseases, atherosclerosis and metabolic conditions [5, 6].

Inflammation is orchestrated by a network of signaling molecules including various immune cell. Cytokines have both inflammatory and anti-inflammatory response. Thus, IL-6, tumour necrosis factor (TNF), and IL-18 are pro-inflammatory cytokines which initiate and promote inflammatory processes [7]. Whereas IL-10, inflammatory receptor agonist (IRA), and transforming growth factor (TGF) are examples of anti-inflammatory cytokines that adversely affect these processes (Figure 1). Subsequently, prostaglandins, neutrophils and macrophages, chemokines are also act as chemical messengers to regulate the immune response. These molecules attract immune cells to the site of inflammation, promote their activation, and coordinate the overall immune response [8].

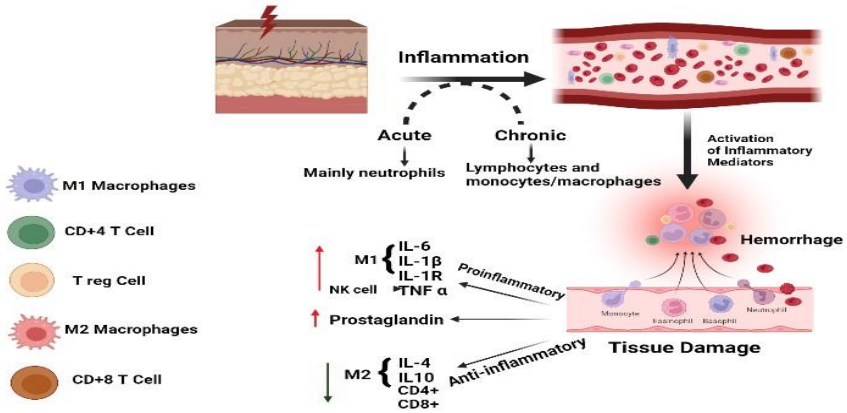


Figure 1. Various inflammatory mediators which regulate inflammatory responses

Currently for the management of the inflammation widely used synthetic drug out of 5% prescribe medicine NASIDS (Non-steroidal anti-inflammatory drugs) [9] like Diclofenac, ibuprofen [10] which inhibits cyclooxygenase (COX) and prostaglandin, its control the release of interleukin (IL) 6 and IL-10 by triggering cAMP and it inhibits many inflammatory mediators [11]. There are many adverse drug reactions of anti-inflammatory drugs. Recently, more emphasis on the examination of medicinal plants to discover new component and validate their traditional uses for the control of inflammatory diseases and for the development of safer and efficient anti-inflammatory agents [12].

In recent years research on the anti-inflammatory properties of the various flavonoids has increased. Flavonoids are a class of organic compound with multiple phenolic structures [13]. The major source of flavonoids are fruits and vegetables. Several studies have demonstrated that flavonoids have many pharmacological properties like anti-inflammatory, antiviral, antioxidant, antiallergenic antineoplastic activities [14, 15]. Flavonoids such as ferulic acid, quercetin, rutin, hesperidin has properties to counteract inflammatory cytokines, which modifying inflammation-related pathways, and controlling inflammatory diseases [16, 17].

Network pharmacology has already emerged as a valuable and indispensable tool for the in-depth study of traditional Indian medicine, contributing to its integration with modern medicine and promoting the development of innovative and effective therapies. network pharmacology has developed into a frequently used analytic technique, promoting the development of the areas of systems biology

and pharmacology [18, 19]. It helps to discovered new mechanism of action towards multiple target and diseases with the help of protein and gene interaction with bioactive molecules.

Molecular docking is most popular bioinformatics modelling in rational drug discovery and structure-based drug design. molecular docking is a computational tool which help to visualizes virtual interaction between protein-ligand molecules [20]. The docking technique's outputs may be used to estimate the binding energy, free energy, and stability of complexes [21].

MATERIALS AND METHODS

1. Selection of bioactive compounds

Flavonoids are the secondary metabolites responsible for an anti-inflammatory potential. Therefore, ferulic acid, quercetin, rutin and hesperidin were selected for the study as these flavonoids are most abundant in maximum of the plant's families.

2. ADME-T Analysis of Bioactive Compounds

In the context of network pharmacology, ADME-T (absorption, distribution, metabolism, excretion, and toxicity) study is a crucial component for the selection of bioactive compound. The Swiss ADME (http://www.swissadme.ch/) and ADMET 2.0 (https://admetmesh.scbdd.com/) online web tool were used to obtain ADME- T properties of compounds [22, 23].

3. Screening of Active Phytoconstituents for Target Predication

The phytoconstituents were screened for its possible target using Swiss target predication (http://www.swisstargetprediction.ch/) and uniport Id was collected from uniport database (https://www.uniprot.org) [24, 25].

3.1 Establishment of a Database of Inflammatory Gene Target

Genecard database (https://www.genecards.org/) online tool was used for the searching of the target for the inflammation [26]. Multiple targets were identified and duplicate targets were removed and processed for Venn diagram construction.

3.2 Construction of Venn Diagram

Venn diagram web tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) generally give information about common targets involved in the drug and disease, based on which one can predict most common targets associated with disease and drug [27].

3.3 Construction of Protein-Protein Interaction

To clear the visualization and understand the molecular mechanism of the targets, it is important to study the Protein-Protein interaction (PPI) of the target genes. The target genes of the corresponding components will be subjected to STRING v12.0 (https://string-db.org/) to visualize and construct the PPI network [28]. The excel sheet of common target gene was upload into string data base where "*Homo sapiens*" species was selected, and medium confidence and "FDR stringency" was set at "medium 5%" in "required score" and construct PPI network and further screened for core genes [29].

3.4 GO Function and KEGG Enrichment Analysis

Gene ontology (GO) enrichment analysis was carried out to analyze the target proteins and KEGG analysis was carried to determine the signaling pathway [30]. David database (https://david.ncifcrf.gov/) was utilized to set the analysis condition "official gene symbol" "*Homo sapiens*" for the KEGG pathway enrichment study and GO function analysis of the inflammatory targets [31]. The pathway was examined in biological process (BP), responsible for inflammatory effect and standard was set as $P< 0.05$. The top 10 genes with relatively minimum P value were screened out in cellular component (CC) and molecular function (MF) respectively. BP, CC, MF data was analyzed and mapped with the help of bioinformatics analysis and visualization of cloud platform online tools (https://www.bioinformatics.com.cn/en). From the analysis of KEGG pathway, "enrichment bar diagram" was fashioned to visualize inflammatory conditions [32].

4. Molecular Docking

Molecular docking is a computational technique used to predict the binding orientation and affinity of a small molecule (ligand) to a target protein or receptor [33]. It plays a crucial role in drug discovery and design by aiding in the identification of potential lead compounds [34]. Using PDB database (https://www.rcsb.org/) we retrieve molecular structure file of the target protein. Autodock vina (version 1.5.7) software used for molecular docking. Hydrogen atoms was added to assign appropriate charges to the protein structure [35]. PyMOL 2.3.0 software was used to download original ligands of target proteins and Pubchem (https://pubchem.ncbi.nlm.nih.gov/) was used to download computational structure files of ligand. Sequentially, Target protein prepared with Autodock vina, target protein rang and location were set for inhibitor after applying grid box to standard compound [36]. The flow chart of whole process of study was depicted in Figure 2.

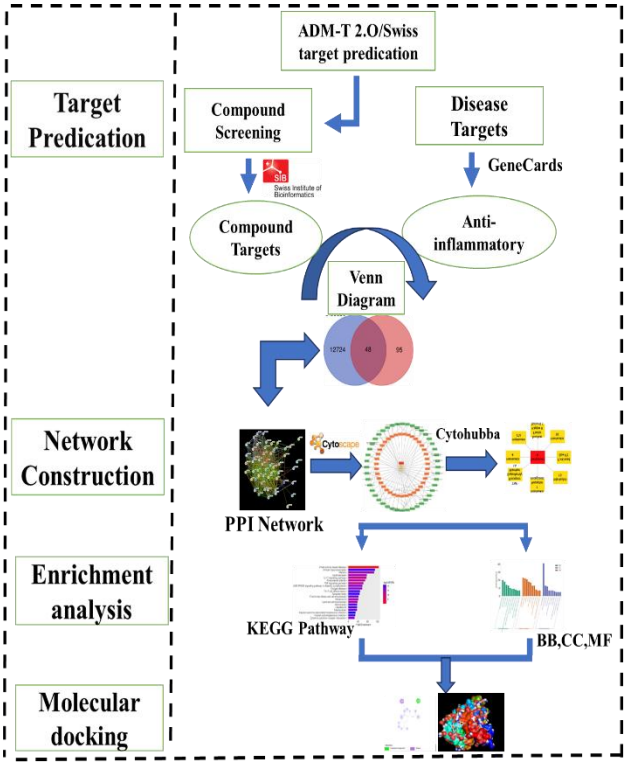


Figure 2. Flow chart of study to explore a potential target of flavonoids for anti-inflammatory activity

RESULTS

Physicochemical parameters and ADMET Analysis

The physicochemical properties and pharmacokinetics parameters of bioactive compounds was examined and discussed in Table 1. Lipophilicity, absorption, distribution, Metabolism, excretion, toxicity of bioactive compound was classified into eight different groups. The oral bioavailability chart of the bioactive compound is mentioned in Figure 3. HIA and BBB penetration are essential parameters that are evaluated during the drug development process. The topological polar surface area (TPSA) is commonly used metric for optimizing a drugs ability to permeate cell membrane. TPSA of CNS drugs usually lower than the non-CNS drugs. Drugs with a lower TPSA (generally below 90 A^2) tend to permeate the BBB more easily, allowing them to reach the brain and exerts

their therapeutic effects. Ferulic acid more easily cross the BBB than other flavonoids as it shown lower TPSA (66.76 Å²). Similarly, the BBB penetration ability of flavonoids is in the range of ferulic acid > quercetin > hesperidin > rutin. These parameters help in assessing the absorption characteristics of a drug and its potential effects on the central nervous system.

Table 1: ADMET information of Ferulic acid, Quercetin, Rutin, Hesperidin

Properties	Parameters	Ferulic acid	Quercetin	Rutin	Hesperidin
Physiochemical	MW[a](g/mol)	194.18 g/mol	302.24 g/ml	610.52 g/mol	610.19
	Rotatable bonds	3	1	6	7
	Fraction Csp3	0.10	0.00	0.44	0.536
	Molar Refractivity	51.63	78.03	141.38	0.54
	TPSA	66.76 Å²	131.36 Å²	269.43 Å²	234.29 Å²
Lipophilicity Log Po/w	iLOGP	1.62	1.63	1.58	2.60
	XLOGP3	1.51	1.54	-0.33	-0.14
	WLOGP	1.39	1.99	-1.69	-1.48
	MLOGP	1.00	-0.56	-3.89	-3.04
	SILICOS-IT	1.26	1.54	-2.11	-1.55
	Consensus Log Po/w	1.36	1.23	-1.29	-0.72
Absorption	GI absorption	High	High	Low	Low
	skin permeation	-6.41 cm/s	-7.05 cm/s	-10.26 cm/s	-10.12 cm/s
Distribution	PPB	89.754%	95.496%	83.811%	77.64%
	VD	0.339	0.579	0.754	0.395
	BBB Penetration	Yes	Yes	No	Yes
Metabolism	CYP1A2 inhibitor	No	Yes	No	No
	CYP2C19 inhibitor	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No
	CYP2D6 inhibitor	No	Yes	No	No
	CYP3A4 inhibitor	No	Yes	No	No
Execretion	Rate of clearance	7.480	8.284	1.489	1.489
Toxicity	hERG Blockers	0-0.1	0-0.1	0-0.1	0-0.1
	Hepatotoxicity	0.3-0.5	0-0.1	0-0.1	0-0.1
	AMES Toxicity	0.1-0.3	0.5-0.7	0.3-0.5	0.3-0.5
	FDAMDD	0-0.1	0.3-0.5	0-0.1	0-0.1
	Skin Sensitization	0.9-1.0	0.9-1.0	0-0.1	0-0.1
	Carcinogenicity	0.3-0.5	0-0.1	0.7-0.9	0.7-0.9
	Eye Irritation	0.9-1.0	0.9-1.0	0-0.1	0-0.1

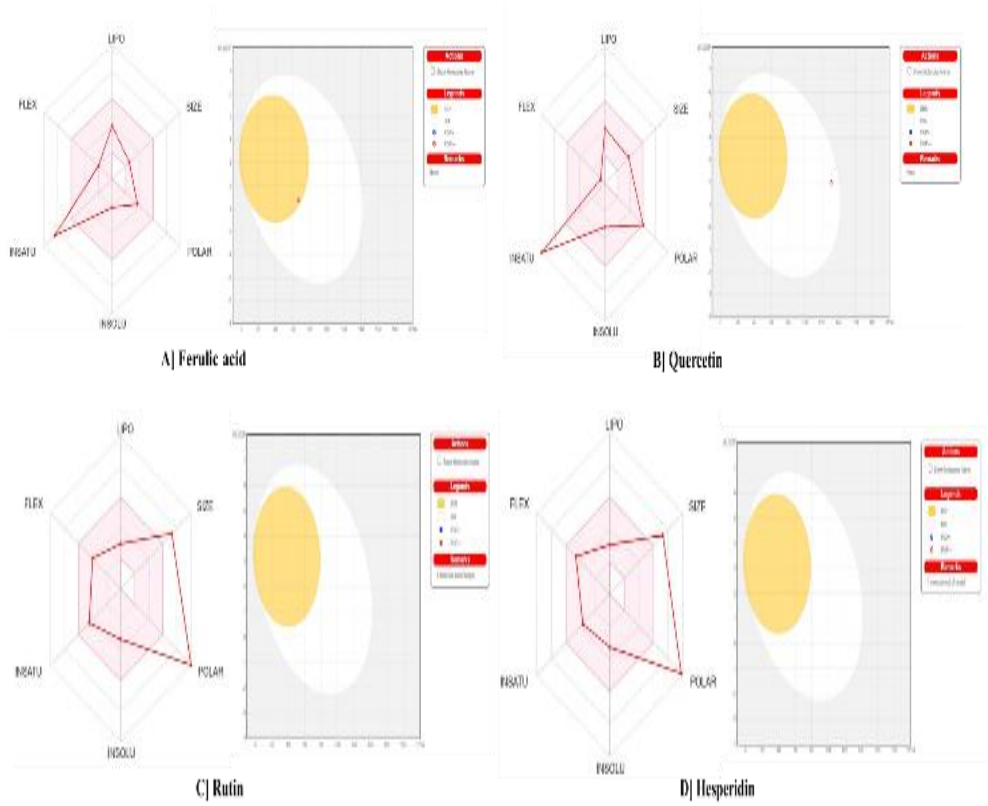


Figure 3. Bioavailability chart for bioactive compounds. The red line indicates oral bioavailability properties and the pink region represents the physicochemical space for oral bioavailability

Acquisition of Target for Inflammation/ Construction of Venn Diagram

There are about 12727 targets for inflammation as per Genecard database. While 97 diseases are associate with ferulic acid, 96 with quercetin and rutin, 95 with hesperidin as per Swiss Target Predication. Out of this 45 common target for Ferulic acid, 55 for Quercetin, 46 for Rutin, and 48 for Hesperidin was observed by Venn diagram.

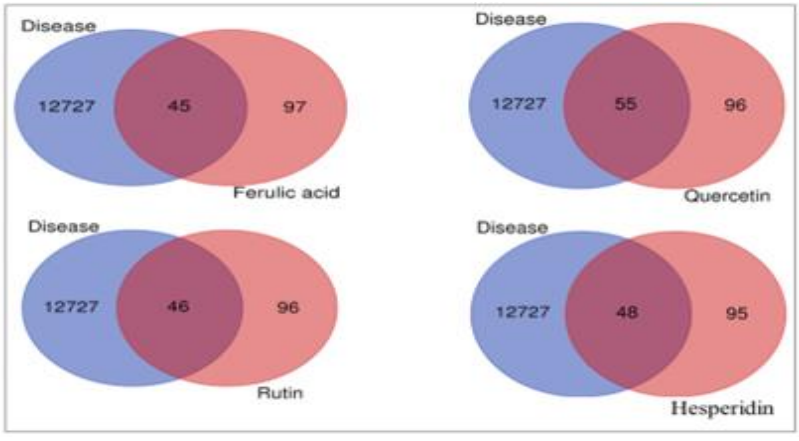


Figure 4. Common target analysis of phytoconstituents with disease targets. The blue color denotes the disease targets and red colour represents the drug targets and the intersection points of circle represents the common targets

GO and KEGG Enrichment analysis

The target genes of the common intersection were examined using the Database for Annotation, Visualization, and Integrated Discovery (David database's), where we performed Gene ontology (GO) enrichment analysis (Figure 5) and Kyoto Encyclopedia of Gene and Genomes (KEGG) to gather the corresponding biological functions and processes (Figure 6). GO enrichment analysis was mainly involved in GO biological process (BP), GO cellular component (CC), and the GO molecular function (MF) these terms were plotted using Bioinformatics tool for the analysis and visualization of data.

Through analysis it was found that the BP of anti-inflammatory effect of ferulic acid, quercetin, rutin and hesperidin mainly focused on GO:1900407~ regulation of cellular response to oxidative stress, GO:0019221~cytokine-mediated signaling pathway, GO:0006954~inflammatory response, GO:0045087~innate immune response etc. CC focused on GO:0005886~plasma membrane, GO:0005615~extracellular space, GO:0005576~extracellular region etc, MF is mainly supplemented in GO:0005515~protein binding, GO:0042803~identical protein binding etc. Analysis of BP, CC and MF data are depicted in F**igure 5**. From the BP analysis it was postulate that anti-inflammatory response of hesperidin was higher when compared with ferulic acid, quercetin and rutin. In CC analysis involvement of extracellular space was observed with all phytoconstituents whereas; in MF analysis most of the phytoconstituents depicted high protein binding when compared with other GO terms associated with MF.

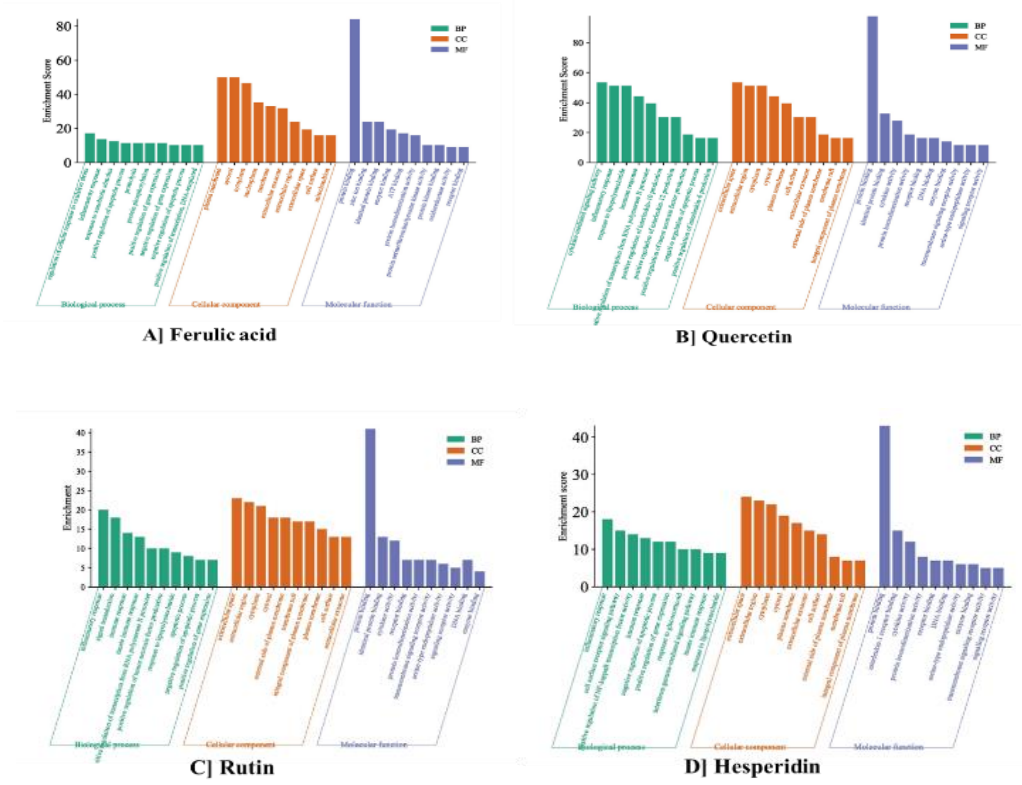


Figure 5. GO enrichment analysis of common targets of the Ferulic acid, Quercetin, Rutin and Hesperidin against inflammation

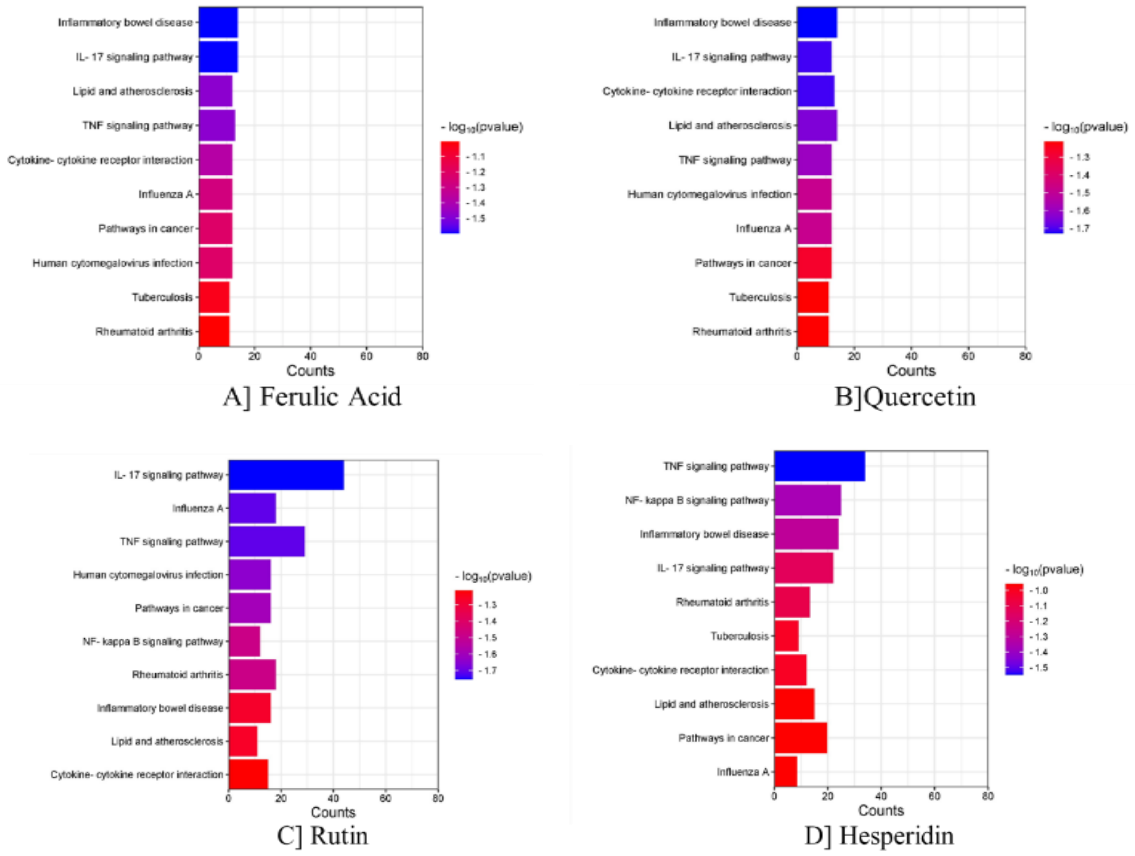


Figure 6. KEGG function analysis

David database was used to analyze the function of KEGG pathway. In figure 6 (A, B, C & D) from the data it was observed that involvement of various pathways those were associated with inflammation among them 74 pathways for ferulic acid; 82 pathways for quercetin; 70 pathways for rutin and 73 pathways for hesperidin. From these data we selected top 10 pathways those were highly associated with inflammation for bar plot analysis. In present analysis P<0.05 were selected as a level of significance; and smaller the P value higher the anti-inflammatory effect.

PPI Network Construction and Analysis

PPI network used to identify the relationship between common targets of bioactive compound with help of string database. All common targets of bioactive compounds showed protein-protein interaction. We got 48 nodes and 568 edges for ferulic acid, 53 nodes and 635 edges for quercetin, 42 nodes and 480 edges for rutin and 43 nodes and 448 edges for hesperidin. PPI network showed the P value < 1.0 e-16 which showed better anti-inflammatory response (Figure 7). Illustration and visualization of PPI network was done by using Cytosacpe whereas; simplification and analysis of network was done by Cytohubba. The top 10 key genes of ferulic acid, quercetin, rutin and hesperidin was shown visually (Figure 8) according to the network, as follows: Ferulic acid (IL17A, IL4, IL13, IFNG, IL1A, IL1RN, NFKB1, IL18, PRTN3, TNFRSF1A) Quercetin (IL1RN, IFNG, MAPK14, PTK2B, GLO1, PDE5A, TLR4, IL10, IRF5, IL6) Rutin (IL17A, IL10, FOXP3, MPO, MMP9, FAS, ICAM1, TNFAIP3, ILRUN, RIPK1) and Hesperidin (NFKB1, FOXP3, IL10, IL17A, IL6, IFNG, IL1RN, TLR2, TLR4, PTGS2).

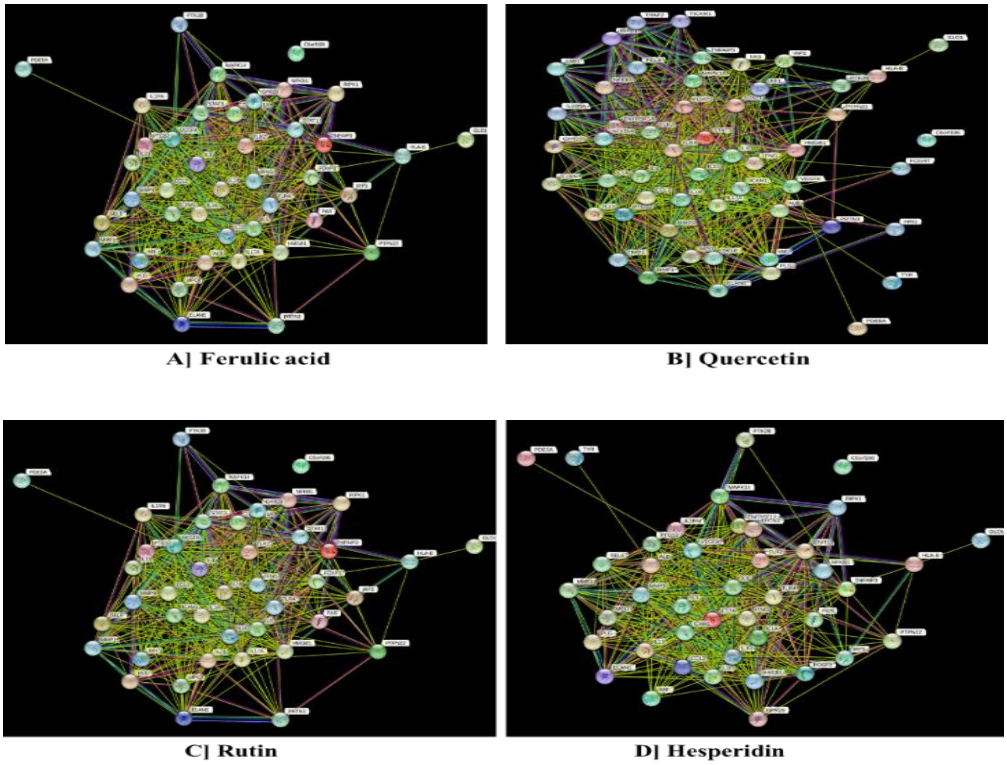
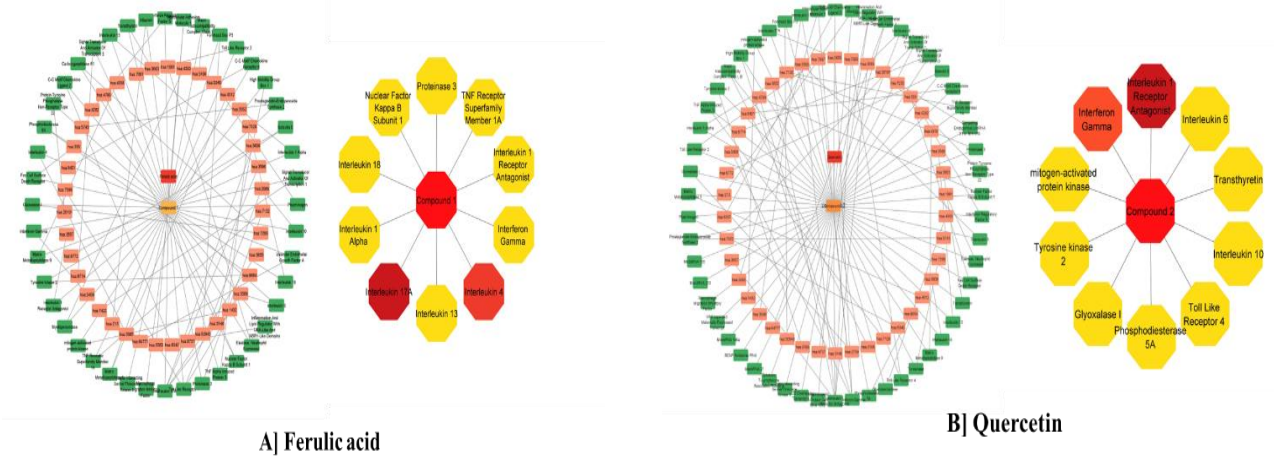


Figure 7. PPI network showing association of nodes and edges responsible for inflammation; these targets were used for computational modelling.



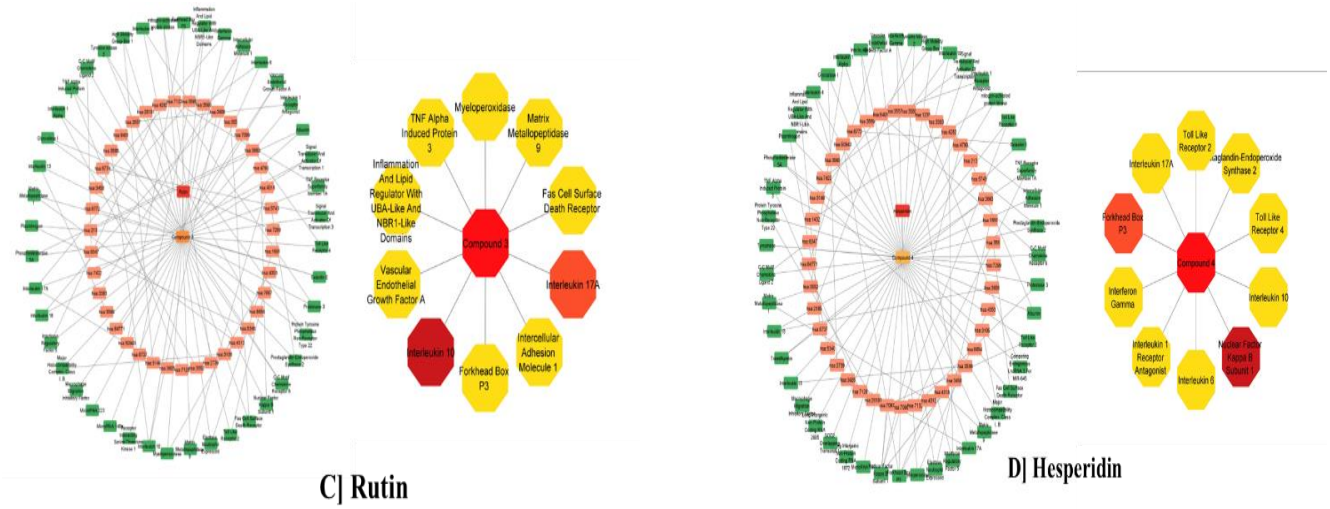


Figure 8. Compound-target pathway network of ferulic acid, quercetin, rutin and hesperidin are represented in fig A, B, C and D and the involvement of degree of interaction between top 10 targets with colors; yellow < orange < brown.

Molecular Docking

After applying Hubba to top 10 targets we identified two proteins from each active compound for molecular docking. On the basis of these, we selected IL 17A and IL4 as target for molecular docking of ferulic acid, IL1RN and IFNG for quercetin, IL17A and IL10 for rutin, NFKB1 and FOXP3 for hesperidin. These targets were selected because they show highest degree of interaction between respective drug and target (Figure 8).

The binding energy in molecular docking refers to the strength of the interaction between a ligand and a receptor. It also quantifies the stability of the complex formed between the ligand and the receptor. The binding energy is typically expressed in terms of a negative value, indicating the favorable nature of the interaction. If negative binding energy is larger than ≤ - 0.5 kcal/mol then stronger binding affinity between the ligand and the receptor. Then Protein-ligand interaction was performed for the selected bioactive compounds. Where, molecular docking results of all four-compound shown significant binding at the pocket site of the protein and Diclofenac was considered as standard control (Table 2).

Quercetin shown less binding free energy with IL1RN (-8.8) and -6.1 against the IFNG receptor than the diclofenac (-7.8 and -5.5 respectively) which indicate significant binding of quercetin and more potent effect than the standard drug (Table 2). Similarly, molecular docking score of rutin [against IL17A (-7.1) & IL10 (-7.7)] and hesperidin [against NFKB1 (-9.7) & FOXP3 (-9.4)] was found to be less than the standard drug diclofenac as displayed in Table 2. It indicates that all the selected flavonoids revealed potent anti-inflammatory potential against various targets of inflammation. Ferulic acid revealed anti-inflammatory potential by binding with IL17A and IL4 with docking score of -5.7 and -5.2 respectively as shown in Figure 9. Binding ability of quercetin, rutin and hesperidin was found to be higher for respective targets than the standard drug and presented in Figure 10, 11 and 12.

Table 2: Molecular binding energy of selected bioactive compounds against various targets

Targets (PDB ID)	Binding free Energy (Kcal/mol)				
	Diclofenac	Ferulic acid	Quercetin	Rutin	Hesperidin
IL17A (2VX)	**-7.0**	-5.7	-	-	-
IL4 (1ITL)	-6.2	-5.2	-	-	-
IFNG (7X45)	-5.5	-	**-6.1**	-	
IL1RN (1G0Y)	-7.8	-	**-8.8**	-	-
IL17A (1ITL)	-6.1	-	-	**-7.1**	-
IL10 (1INR)	-6.2	-	-	**-7.7**	-
NFKB1 (1K3Z)	-6.5	-	-	-	**-9.7**
FOXP3 (7TDW)	-7.0	-	-	-	**-9.4**

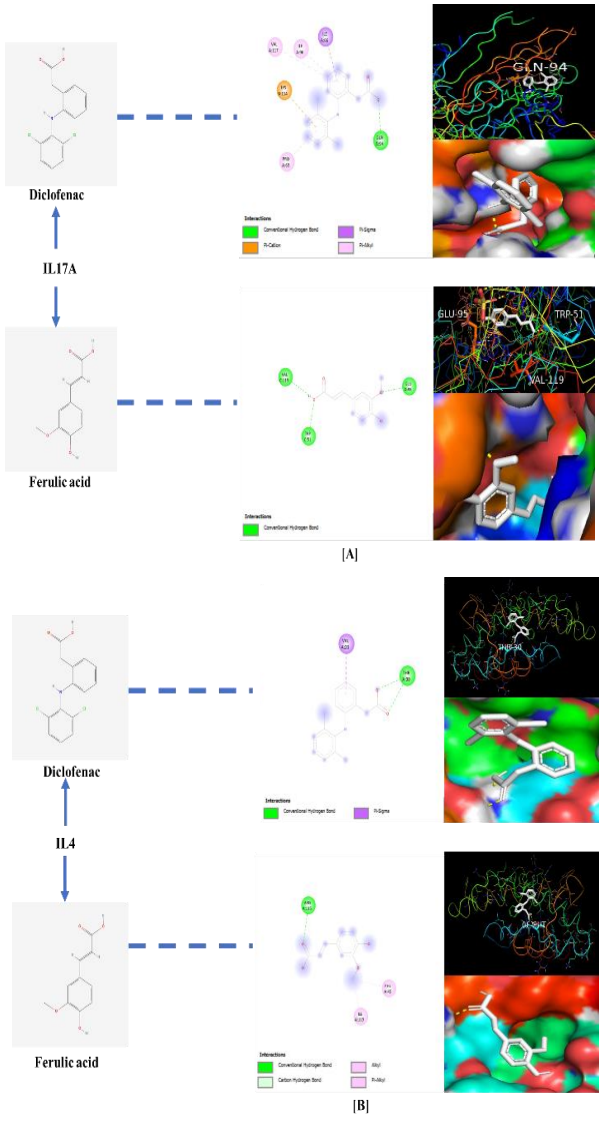


Figure 9. Binding free energy of diclofenac, ferulic acid against IL17A and IL4)

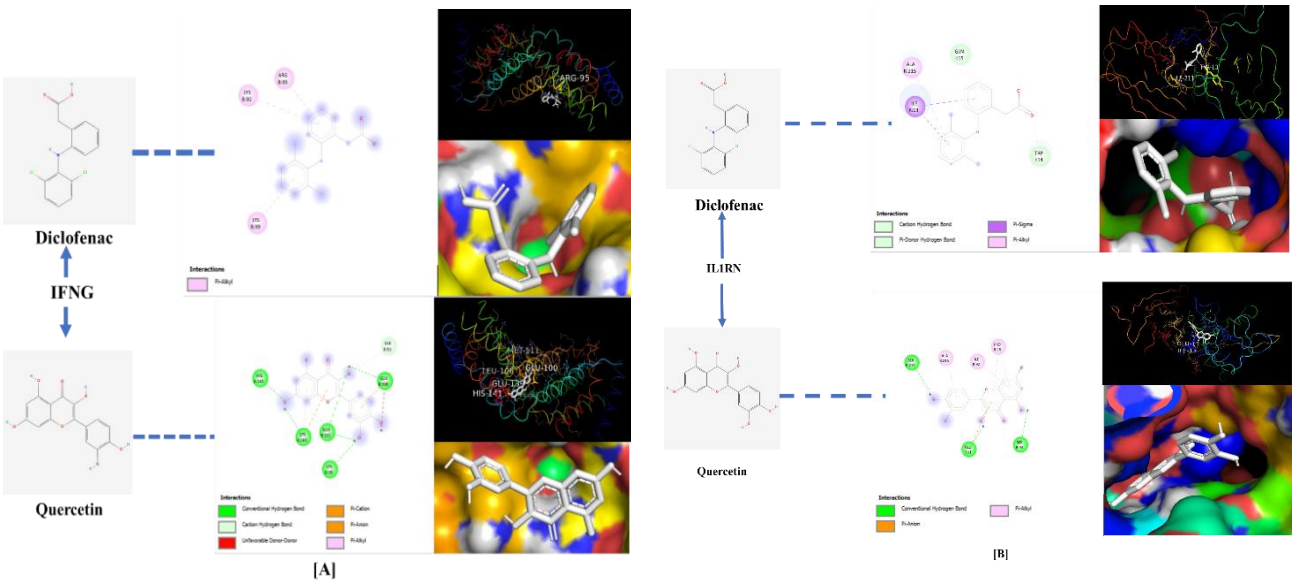


Figure 10. Molecular docking score of quercetin and diclofenac against IFNG and IL1RN

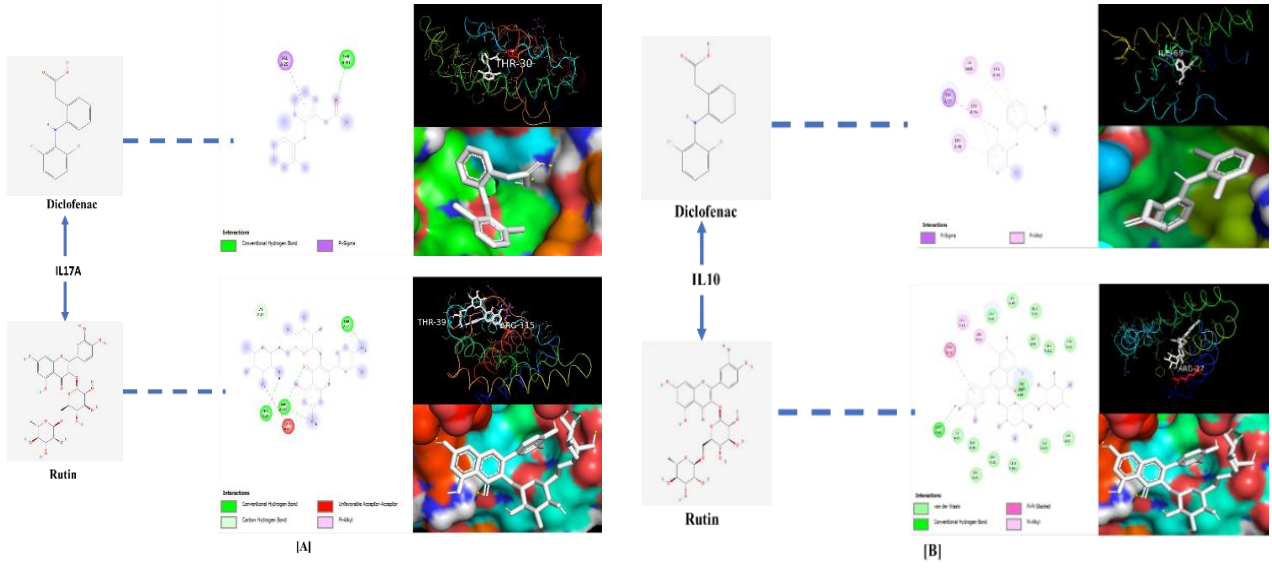


Figure 11. Binding free energies of protein (IL17A, IL10) & ligand (Diclofenac, Rutin)

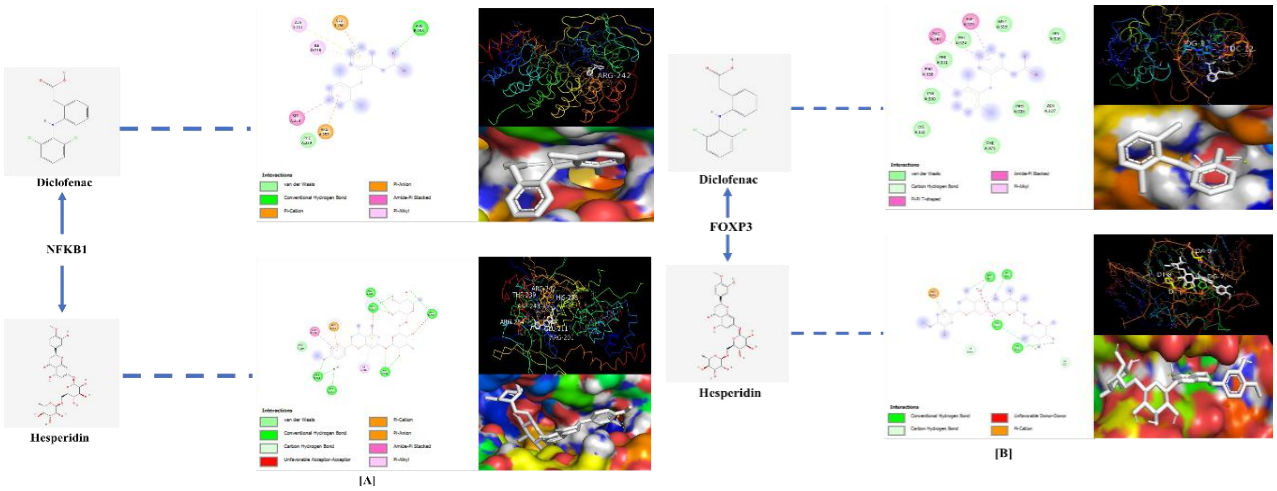


Figure 12. Docking score of protein (NFKB, FOXP3) with diclofenac and hesperidin

DISCUSSION

The inflammatory response is a key component of the body's defense mechanisms, involving a number of cellular and molecular activities. Chronic or excessive inflammation, on the other hand, can help to develop and progress a variety of diseases, including atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, asthma, cardiovascular problems, neurological ailments, autoimmune diseases, and cancer [37, 38]. The use of complementary therapies for treating inflammation is growing by the day, and it frequently goes unreported by physicians [39]. Flavonoids and phenolics are a possible option for developing inflammation-targeted treatments. Their ability to control inflammatory pathways, block pro-inflammatory enzymes, and exert antioxidant effects make them promising candidates for future research and usage in the treatment of inflammatory disorders [40- 42]. As a result, we apply network pharmacology, molecular docking analysis, and ADMET characteristics in this study to evaluate the influence and mechanism of action of flavonoids such as hesperidin, quercetin, rutin, and ferulic acid on inflammation.

Network pharmacology is an interdisciplinary field that combines concepts from pharmacology, network biology, and systems biology to understand the complex interactions between drugs and biological systems [43]. It involves the study of drug-target interactions, signaling pathways, and the overall network of molecular interactions within a biological system. Cytoscape tool was used to analyze the network clusters or modules [44]. By grouping nodes with same characteristics or connectivity patterns, clustering techniques in Cytoscape can expose functional modules inside a network. The anti-inflammatory effect of flavonoids is associated with various pathways, as demonstrated by our investigation of the potential critical nodes IL4, IL 17A, IFNG, IL1RN, IL10, NFKB1, and FOXP3, Figure 8 A, B, C, and D illustrates these findings. Important targets for inflammation include IL4, IL 17A, IFNG, IL1RN, IL10, NFKB1, and FOXP3.

Numerous genes involved in immunological responses are either activated or repressed as a result of this interaction [45]. Inflammatory mediators are produced as a result of the IL17A receptor signaling pathway's primary activation of downstream signaling pathways. The two subunits of the IL-17 receptor, IL-17RA and IL-17RC, are interacting with IL-17A [46]. The activation of the receptor is started when IL-17A attaches to the extracellular domain of IL-17RA. Act1 (sometimes referred to as CIKS), an adaptor protein required for

downstream signaling, is recruited by IL-17RA. Act1 recruits and triggers TNF receptor-associated factor 6 (TRAF6), a crucial signaling protein. Activating TRAF6 activates mitogen-activated protein kinases (MAPKs), which include p38, ERK1/2, and JNK. TRAF6 is an E3 ubiquitin ligase that facilitates the attachment of ubiquitin molecules to target proteins. Numerous transcription factors, including AP-1 (activator protein 1) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), are phosphorylated and activated by these kinases [47]. Genes related to inflammation and immunological responses, including NLRP3, Pro-IL-1β, and Pro-IL-18, are expressed when NF-κB translocates into the nucleus [48].

The immune system's reaction to inflammation and cell death are intricately linked to interferon gamma (IFN-γ) [49]. The Th1 subset is formed when CD4+ T cells differentiate into IFN-γ. Th1 cells are crucial in eliminating intracellular infections because they generate IFN-γ and stimulate macrophages. There are several ways that IL-1RN can signal, including intracellular signaling pathways that are started when IL-1RN binds to the IL-1 receptor on the cell surface. The activation of anti-inflammatory signaling pathways may ensue from this [50]. Interleukin-10, or IL-10, is a significant cytokine that has a role in both immunological control and anti-inflammatory reactions. The IL-10 receptor is composed of two subunits: IL-10R1 and IL-10R2. IL-10R1 is specific to IL-10, while IL-10R2 is shared with other cytokine receptors. IL-10 binds to the IL-10R1 subunit, leading to the recruitment and activation of IL-10R2 [51].

After IL-10 binding, the IL-10 receptor-associated JAK1 and Tyk2 (Janus kinases) are activated. JAKs are enzymes that phosphorylate tyrosine residues on the receptor subunits, initiating downstream signaling events. Activated JAKs phosphorylate and activate STAT proteins, particularly STAT3 [52]. Once activated, STAT3 proteins form dimers and translocate to the nucleus. In the nucleus, STAT3 dimers bind to specific DNA sequences known as STAT response elements (SREs) in the promoter regions of target genes. This binding leads to the transcription of various anti-inflammatory and immunosuppressive genes, including IL-10 itself, SOCS3 (Suppressor of Cytokine Signaling 3), and other downstream effectors. In the nucleus, STAT3 dimers bind to specific DNA sequences known as STAT response elements (SREs) in the promoter regions of target genes. This binding leads to the transcription of various anti-inflammatory and immunosuppressive genes, including IL-10 itself, SOCS3 (Suppressor of Cytokine Signaling 3), and other downstream effectors (Figure 13) [53].

NF-κB regulates the expression of various pro-inflammatory genes, including cytokines (e.g., IL-6, IL-8, TNF-α), chemokines, adhesion molecules, and enzymes involved in the production of reactive oxygen species [54]. These gene products contribute to the recruitment and activation of immune cells, increased vascular permeability, and tissue remodeling, all of which are key features of the inflammatory response. NF-κB pathway is regulated by negative feedback mechanisms to avoid excessive or persistent inflammation. Among these mechanisms are the cytoplasmic sequestration of NF-κB by IκBα resynthesis and the generation of anti-inflammatory molecules (such IL-10) that prevent NF-κB activation [55]. The regulation of immunological responses and the reduction of inflammation are intimately linked to FOX3 signalling. Tregs are the main cells that express FOX3, which is necessary for the formation and suppressive action of these cells. It acts as a master regulator by controlling the expression of various genes involved in Treg differentiation and function [56].

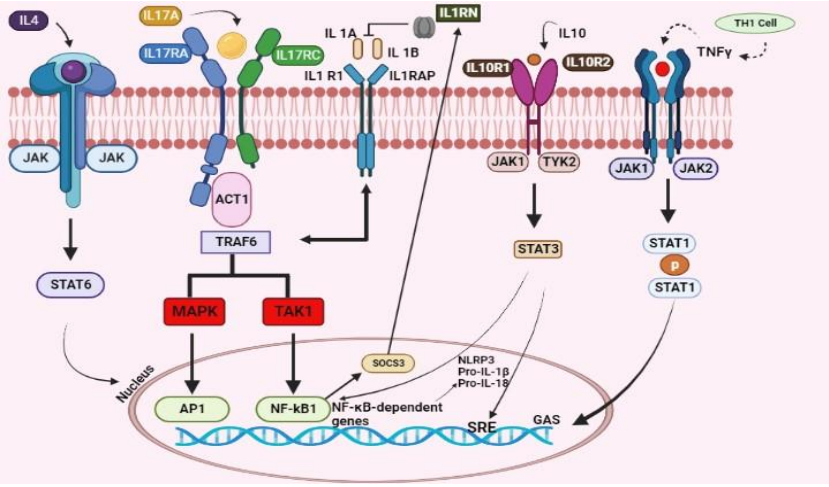


Figure 13. Inflammatory and anti-inflammatory signaling pathways of key targets identified from bioactive compounds

CONCLUSION

The current study reveals that chosen flavonoids (viz., ferulic acid, quercetin, rutin, and hesperidin) have a promising binding with diverse inflammatory targets, revealing their beneficial anti-inflammatory potential. According to the molecular docking results, the ligand exhibits a high affinity for the target protein. These findings provide important insights into the likely method of binding and can be used to validate and optimize the ligand as a potential medicinal agent. This virtual screening of selected flavonoids benefits many scholars in their future studies on the anti-inflammatory potential of natural compounds. It additionally serves as the foundation for future in-vivo and clinical trials on these natural flavonoids.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Abbreviations

IL4: Interleukin-4; **IL6**: Interleukin-6; **IL18** Interleukin-18; **IL10**: Interleukin-10; **IL17A**: Interleukin-17A; **NFKB1**: Nuclear factor kappa B subunit 1; **FOXP3**: Forkhead box protein 3 gene; **IRA**: Inflammatory receptor agonist; **TNF**: Transforming growth factor; **TGF**: transforming growth factor; ADME-T: Absorption, distribution, metabolism, excretion and toxicity; GO: Gene ontology; **KEGG**: Kyoto encyclopedia of genes and genomes; **PPI**: Protein–protein interaction; **BP**: Biological process; **MF**: Molecular function; **CC**: Cellular function; **HIA**: passive gastrointestinal absorption; **BBB**: blood-brain barrier; **JAK/ STAT**: Janus kinase/signal transducers and activators of transcription; **TYK2**: Tyrosine-Kinase 2; **ACT**: Adaptor protein; **AP1**: activator protein 1; **MAPK**: Mitogen-activated protein kinase; **TAK1**: Transforming growth factor-β (TGF-β)-activated kinase 1; **SOCS3**: Suppressor of cytokine signaling 3; **NLRP3**: nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3; **TRAF6**: Tumor necrosis factor receptor associated factor 6.

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استكشاف الأهداف المضادة للالتهابات في الفلافونويدات من خلال الالتحام الجزيئي المتكامل وعلم الأدوية الشبكي

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ملخص

الالتهاب هو استجابة فسيولوجية معقدة مرتبطة بالعديد من الأمراض. أظهرت الفلافونويدات، وهي فئة من المركبات الطبيعية المنتشرة على نطاق واسع في النباتات، خصائص مضادة للالتهابات واعدة. ومع ذلك، تظل آليات عملها الشاملة وأهدافها الجزيئية المحتملة غير قابلة للتعريف. في الدراسة الحالية، استخدمنا نهج علم الأدوية الشبكي جنبًا إلى جنب مع الالتحام الجزيئي للتحقيق في التأثيرات المضادة للالتهابات لبعض الفلافونويدات. في البداية، قمنا بجمع وتنظيم قاعدة بيانات شاملة مثل معلمات وأهداف ADMET من ADME السويسرية وADMET 2.O وتوقع الهدف السويسري. ثم قمنا ببناء شبكة تفاعل بروتين–بروتين لتحديد البروتينات الرئيسية المشاركة في الالتهاب باستخدام قاعدة بيانات السلسلة. بعد ذلك، قمنا بدمج مجموعة بيانات الفلافونويد مع شبكة البروتين لتحديد تفاعلات الفلافونويد–البروتين المحتملة باستخدام Cytosacpe vina. تم إجراء تحليل إثراء GO وKEGG بمساعدة قاعدة بيانات David. تم إنجاز الالتحام الجزيئي من خلال Autodock Vina، وتقييم تقارب ارتباط الفلافونويدات المحددة تجاه البروتينات المستهدفة المحددة. قدم تحليل الالتحام رؤى حول التفاعلات المحددة بين الفلافونويدات والبروتينات المستهدفة، موضحًا الآليات المحتملة الكامنة وراء تأثيراتها المضادة للالتهابات. تعمل المكونات النشطة بيولوجيًا حمض الفيروليك، والكيرسيتين، والروتين، والهسبيريدين على تعديل العديد من العمليات الجزيئية والخلوية ثم تمارس تأثيرات مضادة للالتهابات. من التحليل، شاركت الأهداف الرئيسية في مرض التهاب الأمعاء، ومسار إشارات IL 17، ومسار إشارات TNF، ومسار إشارات بوساطة السيتوكين، والتهاب المفاصل الروماتويدي، والليبوبوليساكاريد وما إلى ذلك. كشفت دراسات الالتحام الجزيئي الإضافية أيضًا أن تقارب الارتباط للفلافونويدات المحددة كان أعلى من تقارب الديكلوفيناك.

الكلمات الدالة: مضاد للالتهابات؛ حمض الفيروليك؛ الهسبيريدين؛ الالتحام الجزيئي؛ علم الأدوية الشبكي؛ كيرسيتين؛ روتين.

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