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Analysis of the active ingredients of Cornus officinalis based on the molecular pharmacology network

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Abstract: *In this study, we investigated the key genes and pathways involved in the treatment of diseases in Cornus officinalis based on the molecular approach of traditional Chinese medicine network. Cornus officinalis is produced in large quantities, is inexpensive, has high nutritional value, and has the efficacy of tonifying the liver and kidney, fixing astringency and stopping sweating. There have been many studies on Cornus officinalis in the past, but most of them only analyze a few active components of Cornus officinalis for specific diseases, and do not comprehensively cover all of its active components. We used the TCMSP database to explore the active components of Cornus officinalis and the corresponding human target genes. Then we used the WebGestalt platform to analyze the KEGG, GO, and other enrichment of human target genes, to obtain the top 10 pathways in terms of enrichment rate to make a histogram and analyze the top three pathways. Then we used String database for protein-protein interaction network analysis to identify a complex network including 29 gene nodes and identify three core nodes corresponding to three core genes. The results showed that there are 20 active ingredients of Cornus officinalis, corresponding to 30 non-redundant human target genes. PPARG, PRKACA and NR3C1 are the three core genes associated with the biological effects of the active ingredients of Cornus officinalis. This study provides guidelines for the potential value of Cornus officinalis in the treatment of many types of diseases and its potential applications in the field of medicine.*

Keywords: *Cornus officinalis, Active components, Database, Pathway*

1. Introduction

Cornus officinalis as a deciduous tree or shrub, has grayish-brown bark, and the branchlets show a fine cylindrical form with a smooth, glabrous surface. The species is mainly found in the Chinese provinces of Shanxi, Shaanxi, Gansu, and Shandong, and also has a natural range in North Korea and Japan. *Cornus officinalis* grows preferably in forest margins or forests at altitudes of 400 to 1,500 meters above sea level. The fruit, commonly known as cornelian cherry or jujube peel, occupies an important position in the field of traditional Chinese medicine, and is regarded as an herb with astringent and strong properties. It is acidic, astringent and slightly warm, and its main effects are to tonify the liver and kidneys, fix astringency and stop sweating. *Cornus officinalis* fruits are rich in 16 amino acids and a variety of essential elements, which play a key role in the pharmaceutical process. Due to its excellent tonic properties, *Cornus officinalis* has been commonly used since ancient times for the treatment of asthma, liver and kidney disorders, and reproductive disorders. Pharmacological studies have revealed that *Cornus officinalis* extracts possess a variety of biological activities such as anti-inflammatory, antioxidant, anti-apoptotic, antidiabetic, neuroprotective and cardiovascular protective activities [1]. In addition, it has been shown that a number of cyclic enol ether terpene glycosides including morroniside, logmalicids, cornusfurosides and cornuside are specific to Cornus corniculatus and are closely related to the corresponding bioactivities [2].

Network molecular pharmacology integrates theories from traditional Chinese medicine, molecular biology, pharmacology, genomics, and other disciplines. It employs network database searches, computer simulations, and other technologies to elucidate the molecular mechanisms of action of the active ingredients in traditional Chinese medicines. This approach, from the perspective of gene expression, offers a powerful tool for the scientific evaluation of the efficacy of these medicines. In

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

exploring the multiple medicinal effects of the traditional Chinese medicine *Cornus officinalis*, the application of molecular networks of traditional Chinese medicines has demonstrated its significant value. A study by Feiqi Huang et al [3] revealed the multi-target and multi-pathway mechanisms of action that may be involved in the treatment of osteoporosis by *Cornus officinalis*. Liu Ping et al [4] focused on the potential mechanism of *Cornus officinalis* in the treatment of depression, and they found that *Cornus officinalis* was able to effectively inhibit the expression of pro-apoptotic factors, such as Bax and caspase-3, while promoting the expression of anti-apoptotic factors, such as NR3C1 and Bcl-2, through neuroactive ligand-receptor interactions, thus showing a broad prospect of *Cornus officinalis* as a potential treatment for depression. Yanjie Qu et al [5], on the other hand, utilized the molecular network of *Cornus officinalis* to elucidate the precise regulation of key aspects of amyloid deposition, apoptosis, autophagy, inflammatory response and oxidative stress by shamrocks through the key signaling pathways, such as PI3K-AKT, MAPK, and so on for the treatment of Alzheimer's disease. However, previous studies have often analyzed the active components of *Cornus officinalis* only for specific diseases and have not been able to comprehensively cover all its potential active components, which deserves further exploration.

The therapeutic mechanism of Chinese medicines does not follow the paradigm of chemical drugs, which are directed at a single component and act on a single target. Although their direct effect on a specific target may be mild, Chinese medicines are able to comprehensively regulate pathological processes through networked biological interactions, thus demonstrating unique and far-reaching therapeutic effects in maintaining the overall balance of the organism and restoring health. For *Cornus officinalis*, the subject of this study, we adopted the molecular network approach of Chinese medicine to systematically screen its active ingredients and further identify the human target genes corresponding to these active ingredients, aiming to construct an exhaustive molecular action network. On this basis, we predicted the possible therapeutic targets of *Cornus officinalis* and hypothesized the potential application value in the treatment of various disease types and in a wide range of medical fields.

2. Methods

2.1. Collection of Active Ingredients of Cornus Officinalis and Prediction of Target Genes

In this study, we used TCMSP database (https://old.tcmsp-e.com/tcmsp.php)[6] , and searched the database for the chemical constituents of *Cornus officinalis*, and screened the obtained active constituents for OB (Oral Bioavailability ≥30%) and DL (Drug Likeness ≥0.18). The corresponding SMILES and 2D structures were obtained, and the 2D structures were saved in sdf format. Subsequently, we sequentially viewed the related targets of the active genes, i.e., we obtained the related target enzymes of the genes, and counted the genes and corresponding enzymes after de-redundancy. Then, we matched the active components with their human host target genes to obtain the non-redundant human target genes as well as the related enzymes.

2.2. Enrichment Analysis of Target Genes

We used WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) (https://www.webgestalt.org/) [7] to enrich the target human target genes in various bioinformatics contexts. We selected the "Gene Set Enrichment Analysis" module and designated "Organism of Interest" as human (homo sapiens). Firstly, this study focused on the KEGG signaling pathway. In the "Functional Database" option, we selected the "KEGG" section under the "pathway" category, and the $P \leq 0.05$ and $FDR \leq 0.05$ were used for the analysis of the KEGG signaling pathways. We screened and counted the top 10 KEGG signaling pathways, and visualized the results by drawing bar charts. Further, we switched from "Functional Database" to "Gene Ontology" and performed the following analysis for "Biological Process noRedundant", "Cellular Component noRedundant" and "Molecular Function noRedundant", respectively. We set $P \leq 0.05$ as the screening threshold, and also ranked the top 10 GO signaling pathways under each category in descending order of enrichment rate, and plotted the histograms. Finally, by selecting "Disease", "chromosomalLocation" and "cell-type" as the "Functional Database", the top 10 GO signaling pathways in each category were filtered and counted, and the histograms were plotted. We explored the enriched characteristics of disease, chromosomal location and cell-type respectively, and the results were also sorted in descending order of the enrichment rate, and plotted in bar charts.

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

2.3. Protein-protein Interaction Network Analysis and Core Gene Screening Analysis

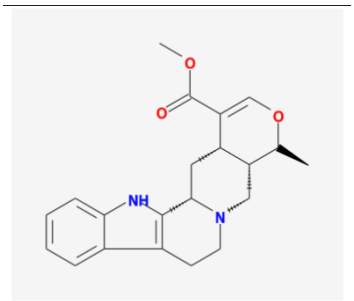
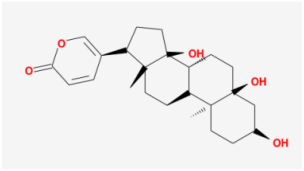
We used the String database (https://string-db.org/) [8] to perform protein-protein interaction (PPI) analysis on the target human target genes. We selected the "Multiple proteins" query mode in the String database, and then imported the target genes in batch, and limited the species option to human (homo sapiens), so as to obtain the output of the PPI network graph. The nodes represent individual proteins and edges represent relationships between proteins. By deeply analyzing the degree distribution of network nodes, we identified three core nodes. We used GeneCards - the human gene database (www.genecards.org) [9] to collect specific information of the above three core genes and analyzed GeneCards Symbol, Gene specific function, NCBI Gene Summary, Three dimensional structures from PDB (representative), AlphaFold (predicted), Subcellular locations from UniProtKB/Swiss-Prot and Pathways by source specific information for summary statistics.

3. Results

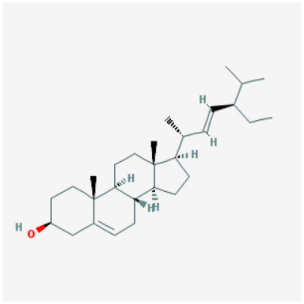
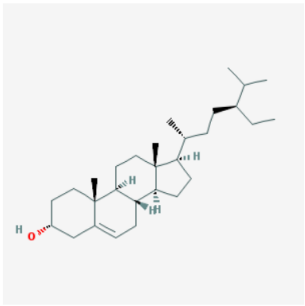
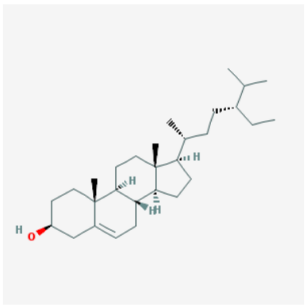
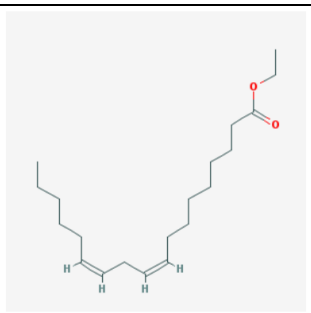
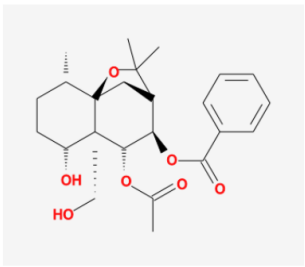
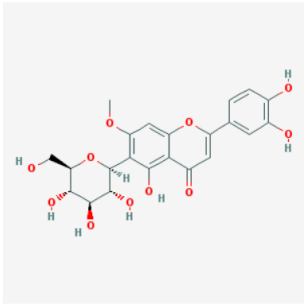
3.1. Collection of Active Ingredients of Cornus Officinalis and ADME Analysis

In this study, a total of 226 active ingredients of *Cornus officinalis* were retrieved from TCMSP database, and then 20 active ingredients were obtained according to the screening criteria of ADME parameters OB ≥ 30% and DL ≥ 0.18 (Table 1). The molecular names, OB values, DL values, structural formulas and Cid numbers of the compounds of the 20 active ingredients are specifically given in Table 1. Among the OB values of these 20 active ingredients, the maximum value was 69.99% (corresponding to the active ingredient Telocinobufagin), the minimum value was 30.25% (corresponding to the active ingredient gallic acid-3-O-(6'-O-galloyl)-glucoside), and the average value was 42.09%. The DL values of these 20 active ingredients had a maximum value of 0.82 (corresponding to the active ingredient lanosta-8, 24-dien-3-ol,3-acetate), a minimum value of 0.19 (corresponding to the active ingredient Ethyl oleate (NF) with Mandenol), and a mean value of 0.55. 13 of these 20 active ingredients had compound Cid numbers and 7 did not have compound Cid numbers. Afterwards, we matched the active ingredients with their human host target genes, and a total of 14 active ingredients in *Cornus officinalis* were found to correspond to 76 host-related target genes. Among them, sitosterol, beta-sitoster, Hydroxygenkwanin, and Tetrahydroalstonine also corresponded to 10 target-related genes. The remaining active ingredients corresponded to less than 3 target genes, leaving a total of 30 non-redundant human target genes after target gene deletion of duplicates.

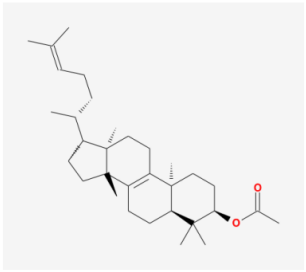
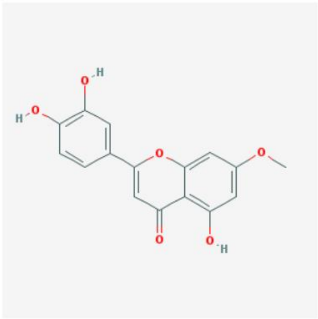
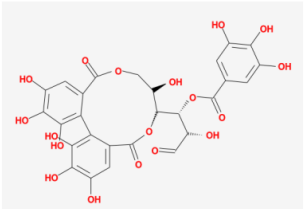
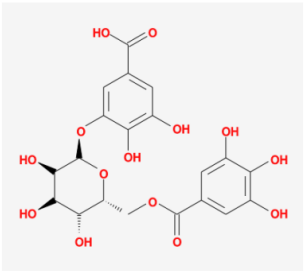
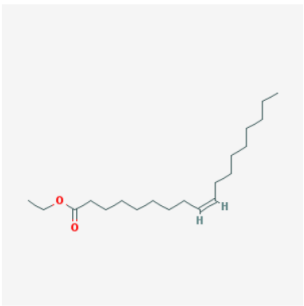
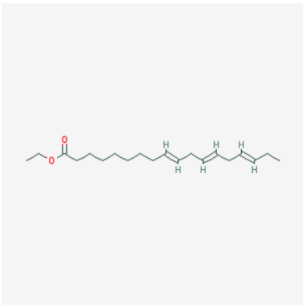
Table 1: Active ingredient information of Cornus officinalis

Molecule Name	OB%	DL	Structure	Pubchem Cid
Tetrahydroalstonine	32.42	0.81		N/A
Telocinobufagin	69.99	0.79		N/A

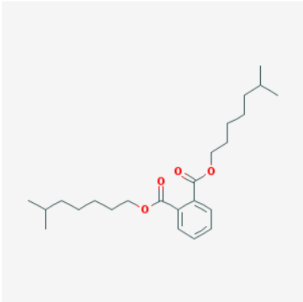
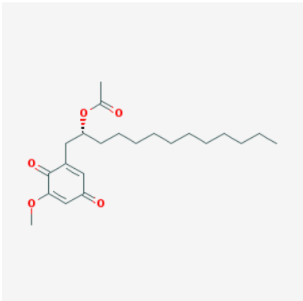
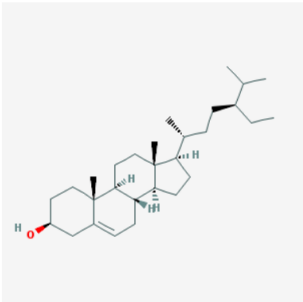
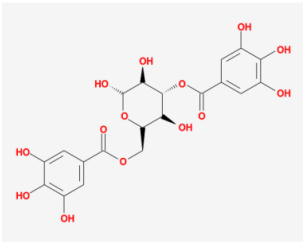
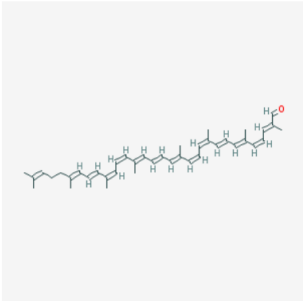
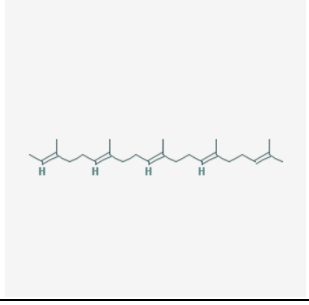
ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

Stigmasterol	43.83	0.76		5280794
sitosterol	36.91	0.75		12303645
poriferast-5-en-3beta-ol	36.91	0.75		457801
Mandenol	42.00	0.19		5282184
malkangunin	57.71	0.63		N/A
Leucanthoside	32.12	0.78		442659

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

lanosta-8,24-dien-3-ol, 3-acetate	44.30	0.82		N/A
Hydroxygenkwanin	36.47	0.27		5318214
gemin D	68.83	0.56		N/A
gallic acid-3-O-(6'-O-galloyl)-glucoside	30.25	0.67		N/A
Ethyl oleate (NF)	32.40	0.19		5363269
Ethyl linolenate	46.10	0.20		6371716

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

Diop	43.59	0.39		33934
Cornudentanone	39.66	0.33		46191017
beta-sitosterol	36.91	0.75		222284
3,6-Digalloylglucose	31.42	0.66		N/A
3,4-Dehydrolycopen-16-al	46.64	0.49		5316458
2,6,10,14,18-pentamethylicosa-2,6,10,14,18-pentaene	33.40	0.24		5366013

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

3.2. Human Target Gene Enrichment Analysis

The results of KEGG signaling pathway enrichment analysis revealed for us the central role of the target genes in the complex regulatory network in the organism. We identified the top ten KEGG signaling pathways closely related to the 30 human target genes screened (Figure 1, Table 2). Among them, the pathway regulating lipolysis in adipocytes topped the list with an enrichment rate of 23, suggesting the critical role of the target genes in fat metabolism, especially lipolysis. Specifically, the genes involved in this pathway include ADRB2 (adrenergic receptor β2), which plays a central role in regulating adipocyte response to catecholamine hormones; PRKACA (protein kinase A catalytic subunit α), one of the key enzymes regulating a variety of cellular processes, which also plays an important role in lipolysis; PTGS1 (prostaglandin endoperoxidant synthase 1) and PTGS2 (prostaglandin endoperoxide synthase 2), both of which are involved in the synthesis of prostaglandins, which play an important role in the regulation of both inflammation and lipid metabolism. This is closely followed by the salivary secretion pathway (Enrichment ratio = 14.4), which reveals the potential role of the targeted genes in the maintenance of oral health and digestion. Genes in this pathway include ADRB2 (adrenergic receptor β2), CALML5 (calmodulin-like protein 5), which may be involved in the regulation of intracellular calcium ion homeostasis; CHRM3 (cholinergic receptor M3), one of the major receptors for acetylcholine, which plays a key role in the secretory activities of the salivary glands; and PRKACA, whose wide-ranging role in cellular signaling is alsoreflected in this pathway. The cholinergic synaptic pathway (Enrichment ratio = 11.8), on the other hand, reveals an important role for target genes in neurotransmission and synaptic plasticity. Genes in this pathway include CHRM1 (cholinergic receptor M1), which is also sensitive to acetylcholine; CHRM3 (cholinergic receptor M3); PIK3CG (phosphatidylinositol-3-kinase catalytic subunit γ), which is a key component of the PI3K/Akt signaling pathway and is involved in the regulation of a variety of cellular functions; and PRKACA, which, by its catalytic activity, further enriches the complexity of the pathway in neurotransmission and cell response. Pathway's complexity in neurotransmission and cellular response through its catalytic activity.

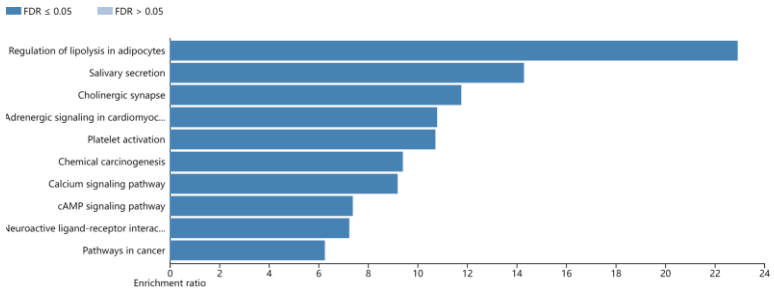


Figure 1: Bar graph of KEGG signaling pathway enrichment analysis

Table 2: Target genes in enriched pathways

Gene Symbol	Gene Name	Pathway
ADRB2	adrenoceptor beta 2	Regulation of lipolysis in adipocytes
PRKACA	protein kinase cAMP-activated catalytic subunit alpha	Regulation of lipolysis in adipocytes
PTGS1	prostaglandin-endoperoxide synthase 1	Regulation of lipolysis in adipocytes
PTGS2	prostaglandin-endoperoxide synthase 2	Regulation of lipolysis in adipocytes
ADRB2	adrenoceptor beta 2	Salivary secretion
CALML5	calmodulin like 5	Salivary secretion
CHRM3	cholinergic receptor muscarinic 3	Salivary secretion
PRKACA	protein kinase cAMP-activated catalytic subunit alpha	Salivary secretion
CHRM1	cholinergic receptor muscarinic 1	cholinergic synapse
CHRM3	cholinergic receptor muscarinic 3	cholinergic synapse
PIK3CG	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit qamma	cholinergic synapse
PRKACA	protein kinase cAMP-activated catalytic subunit alpha	cholinergic synapse

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

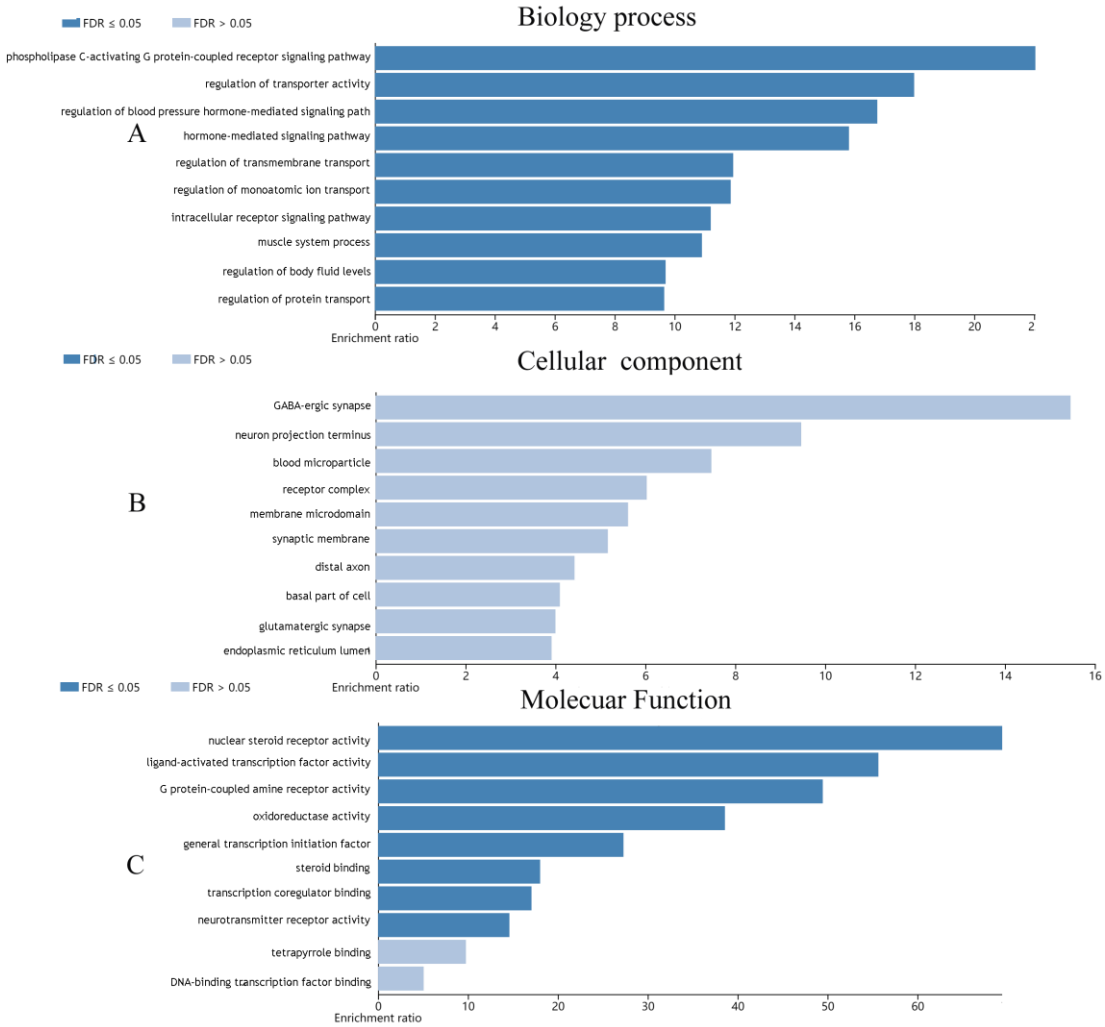


Figure 2: GO enrichment analysis histogram

GO enrichment analysis revealed the important roles of these target genes at the level of biological processes, cellular components and molecular functions (Figure 2). At the biological process level, the phospholipase C-activated G protein-coupled receptor signaling pathway exhibited the highest enrichment ratio (Enrichment ratio = 22), followed by the regulatory pathway for transport activity (Enrichment ratio = 18) and the hormone-mediated signaling pathway for regulation of blood pressure (Enrichment ratio = 16.6). At the cellular component level, the GABAergic synaptic pathway (Enrichment ratio = 15.6) was the most significant, followed by the neuronal secretion endpoint pathway (Enrichment ratio = 9.6) and the blood particle pathway (Enrichment ratio = 7.6). As for molecular function, the nuclear steroid receptor activity pathway exhibited the highest enrichment ratio (Enrichment ratio = 70), followed by the ligand-activated transcription factor activity pathway (Enrichment ratio = 55.5) and the G protein-coupled amine receptor activity pathway (Enrichment ratio = 49.5).

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

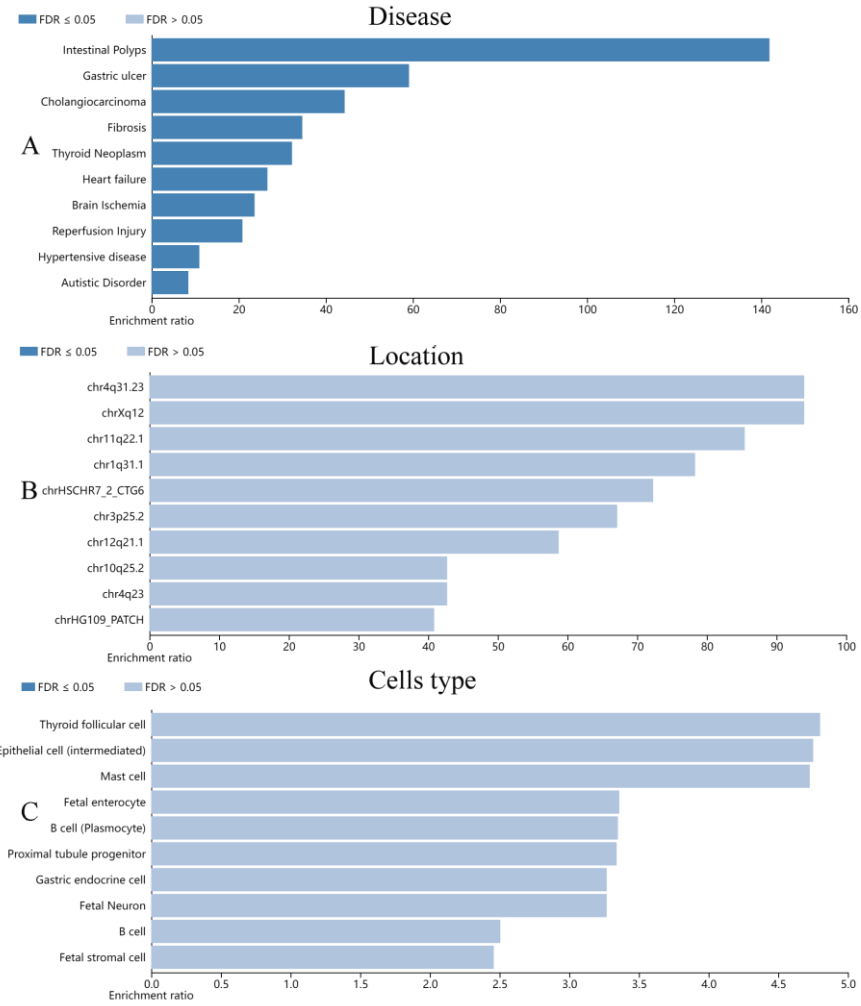


Figure 3: Other enrichment analysis histograms (based on Disease, Hromosome location and Cells type)

In addition, other enrichment analyses provided insights into the role of target genes in disease, chromosomal location, and cell type (Figure 3). Analysis of the disease function database noted that the intestinal polyp pathway had the highest enrichment ratio (Enrichment ratio = 143). In terms of chromosomal location, both the chr4q31.23 pathway and the chrXq12 pathway showed significant enrichment (Enrichment ratio = 94). The cell type function database, on the other hand, revealed the thyroid follicular cell pathway as the most enriched cell type (Enrichment ratio = 4.75), closely followed by epithelial cells (intermediary, Enrichment ratio = 4.7) and mast cells (Enrichment ratio = 4.67). These findings provide new perspectives and insights into understanding the role of target genes in complex biological processes.

3.3. Protein-Protein Interaction Network Analysis

In the protein-protein interaction network (PPI) analysis, we identified a complex network containing 29 gene nodes, which exhibits rich interactions among these gene products through 70 edges (Figure 4, Table 3). The average node degree of the network was calculated to be 4.83, revealing a high density of connections within the network. In addition, the average local clustering coefficient was 0.514, indicating significant local clustering among nodes, which promoted information transfer and functional synergy within the network. Compared with the random network, the expected number of edges of this PPI network was only 19, while the actual number of edges significantly exceeded this value (p-value < 1.0e-16), further emphasizing the non-random and biologically important nature of the network structure.

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

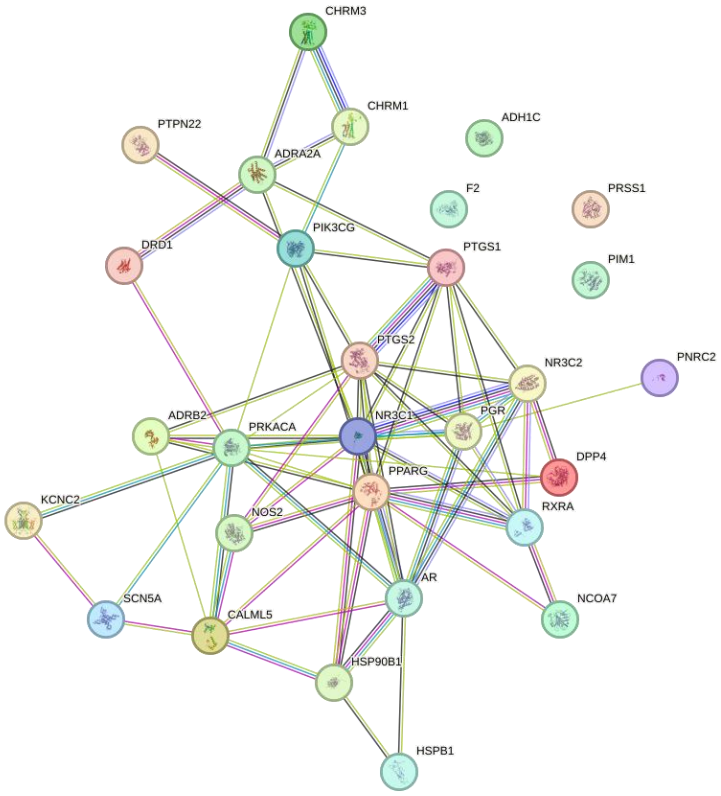


Figure 4: Protein-Protein interaction network

Table 3: Summary of PPI network

Network characteristics	statistics
number of nodes:	29
number of edges:	70
average node degree:	4.83
avg. local clustering coefficient:	0.514
expected number of edges:	19
PPI enrichment p-value:	<1.0e-16

Through an in-depth analysis of the degree distribution of the network nodes, we identified three core nodes, PPARG, PRKACA, and NR3C1, which possessed node degrees of 13, 12, and 12, respectively. It were significantly higher than those of the other nodes in the network, and thus served as key hubs in this PPI network (Figure 5, Figure 6, Table 4). Specifically, the PPARG node represents a key member of the peroxisome proliferator-activated receptor (PPAR) subfamily, which encodes a nuclear receptor that regulates the transcriptional activities of a variety of genes through the formation of heterodimers with retinoid X-like receptors (RXRs) and has a wide range of effects on processes such as cellular metabolism, differentiation, and inflammatory responses. The high connectivity of this node reflects its central position in the regulatory network and may serve as an intersection of multiple signaling pathways. The PRKACA node, on the other hand, is associated with the catalytic subunit α of protein kinase A (PKA), which, as an important intracellular signaling molecule, plays a key role in a variety of cellular processes through its catalytic subunit, including protein phosphorylation, cell proliferation and differentiation. The high connectivity of the PRKACA node suggests its central position in the PKA signaling pathway and may be involved in regulating the activity of multiple downstream targets. Finally, the NR3C1 node encodes the glucocorticoid receptor, as a member of nuclear receptor subfamily 3 group C, it not only directly responds to glucocorticoid signaling and activates the transcription of related genes, but also serves as a regulator of other transcription factors and participates in a complex regulatory network of gene expression. The high connectivity of NR3C1 nodes reveals their central role in physiological processes such as stress response, immune regulation and metabolic homeostasis.

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

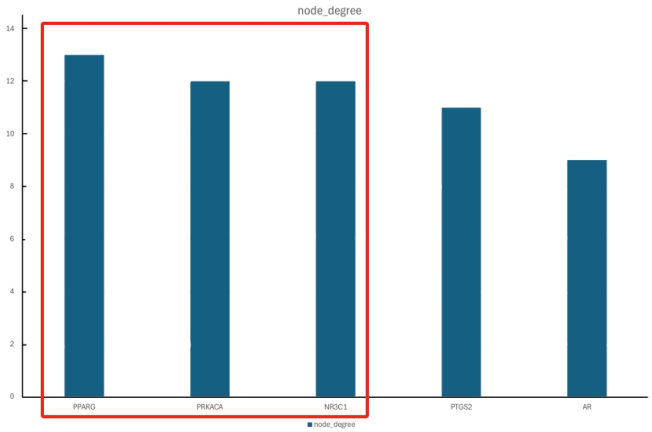
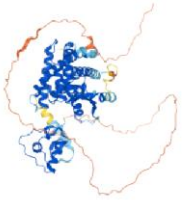
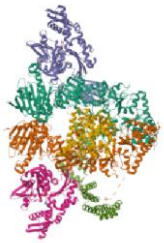


Figure 5: Node degree of three core genes

Figure 6: Major Tissues of three core genes

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

Table 4: Hub gene summary of PPI network

Gene Symbol	PPARG	PRKACA	NR3C1
Gene function	Peroxisome Proliferator Activated Receptor Gamma	Protein Kinase CAMP-Activated Catalytic Subunit Alpha	Nuclear Receptor Subfamily 3 Group C Member 1
NCBI Gene Summary	This gene encodes a member of the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors. PPARs form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate transcription of various genes. Three subtypes of PPARs are known: PPAR-alpha, PPAR-delta, and PPAR-gamma. Additionally, PPAR-gamma has been implicated in the pathology of numerous diseases including obesity, diabetes, atherosclerosis and cancer.	This gene encodes one of the catalytic subunits of protein kinase A, which exists as a tetrameric holoenzyme with two regulatory subunits and two catalytic subunits, in its inactive form. cAMP causes the dissociation of the inactive holoenzyme into a dimer of regulatory subunits bound to four cAMP and two free monomeric catalytic subunits. Four different regulatory subunits and three catalytic subunits have been identified in humans. cAMP-dependent phosphorylation of proteins by protein kinase A is important to many cellular processes, including differentiation, proliferation, and apoptosis.	This gene encodes glucocorticoid receptor, which can function both as a transcription factor that binds to glucocorticoid response elements in the promoters of glucocorticoid responsive genes to activate their transcription, and as a regulator of other transcription factors. This receptor is typically found in the cytoplasm, but upon ligand binding, is transported into the nucleus. It is involved in inflammatory responses, cellular proliferation, and differentiation in target tissues. Mutations in this gene are associated with generalized glucocorticoid resistance.
Three dimensional structures			
Subcellular locations from UniProtKB/Swiss-Prot	Nucleus/Cytoplasm	Cytoplasm/Cell membrane/Membrane/Nucleus/ Mitochondrion	Cytoplasm/Nucleus/Mitochondrion
Pathways	MAPK-Erk Pathway	Actin Dynamics Signaling Pathway AMPK Signaling Pathway G Protein-coupled Receptors Signaling Pathway MAPK-Erk Pathway	Glucocorticoid receptor pathway Mammary gland development pathway - Pregnancy and lactation (Stage 3 of 4) Nuclear receptors meta-pathway Serotonin receptor 4/6/7 and NR3C signaling Sudden infant death syndrome (SIDS) susceptibility pathways Transcription factor regulation in adipogenesis White fat cell differentiation

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

4. Discussion

Cornus officinalis is a deciduous tree or shrub widely distributed in China and some East Asian countries, and its medicinal value has been important in traditional medicine since ancient times. It is also a valuable herb for treating anemia, lumbago, nervousness, heart weakness and other diseases. In recent years, with the deepening of modern pharmacological research, a variety of pharmacological activities of *Cornus officinalis* have gradually been scientifically verified. Experimental evidence has shown that *Cornus officinalis* extracts and their active ingredients exhibit a wide range of biological activities, including but not limited to immunomodulation, blood glucose lowering, antishock, antiarrhythmic, antibacterial, anti-inflammatory, anti-aging, anticancer and anti-AIDS [10]. Particularly notable is its potential in neuroprotection and cardiovascular protection, which provides new possible pathways for the treatment of neurological and cardiovascular diseases [1]. In addition, the role of *Cornus officinalis* in regulating body metabolism and lowering blood lipids has further broadened its clinical applications [11]. In view of such rich pharmacological activities and wide application prospects of *Cornus officinalis*, it is particularly important to deeply explore the material basis of its pharmacological effects and its mechanism of action.

We adopted the emerging interdisciplinary approach of molecular networks of TCM, which integrates the multidisciplinary strengths of pharmacology, molecular biology, and computational biology, aiming to reveal the interactions among the complex components of TCM and their network regulation mechanisms with disease targets, and it is a multidisciplinary cross-disciplinary tool that integrates bioinformatics, pharmacology of TCM, network science, and computer science [12]. By utilizing advanced databases such as TCM System Pharmacology Analysis Platform (TCMSP), GeneCards, CTD, and Drugbank, it can predict the material basis and mechanism of action of Chinese medicines, speculate the active substance components in Chinese medicines, search for the relevant human target genes, construct a network map of the core targets of drug therapy for diseases, and construct a disease-gene-target-drug interactionnetwork and other functions [13]. We systematically analyzed the pharmacological substance basis of *Cornus officinalis* and predicted the potential genes acting on the human body, and also identified the core targets of *Cornus officinalis* active ingredients acting in the human body based on the PPI network structure.This finding not only verifies the scientific basis of *Cornus officinalis* in the treatment of diseases, but also provides a new perspective for a more comprehensive understanding of its pharmacological mechanism of action through the construction of a "multi-component-multi-target-multi-pathway" network model. Although the molecular network pharmacology of TCM is still facing the challenges of imperfect database and lagging update, its systematic and holistic research concepts are highly compatible with the principles of "holistic concept" and "identification and treatment" of TCM [14], which provides a strong support for the modernization and clinical application of TCM. It provides strong support for the modernization research and clinical application of traditional Chinese medicine. Therefore, the molecular network study of *Cornus officinalis* is not only helpful to deepen the understanding of the material basis of its efficacy and the mechanism of its pharmacological action, but also expected to discover new drug targets and optimize the drug combination strategy, which will lay a solid scientific foundation for the modernization, research, development and clinical application of *Cornus officinalis* and its related preparations.

In this study, we systematically searched and identified 20 active components in *Cornus officinalis* with the help of the TCMSP database, covering key compounds such as tetrahydroalpha-octocrystalline, farnesoidal toadstool, ergosterol, sterol and cholest-5-en-3β-ol. These findings not only enriched the chemical composition library of *Cornus officinalis*, but also provided a solid foundation for further exploration of its pharmacological effects. Specifically, the study of Yumei Liao et al [15] revealed the potential role of tetrahydroaldoxine in protecting against OGD/R-induced neuronal damage. On the other hand, the study of Yiliu Shen et al [16] showed that Farfarina toadstool extract exhibited anti-tumor potential in non-small cell lung cancer by inhibiting the STAT3 signaling pathway, further expanding the prospects of *Cornus officinalis* in the field of anti-cancer applications.These studies imply that the multiple active components of *Cornus officinalis* possess cross-field therapeutic value.

Through KEGG signaling pathway enrichment analysis, we found that the human target genes targeted by these active ingredients were mainly enriched in key biological pathways such as adipocyte lipolysis regulation, salivary secretion, and cholinergic synapses, which provide important clues for an in-depth understanding of the pharmacological mechanism of *Cornus officinalis*. (1) Adipocyte lipolysis-regulated pathway: This pathway plays a central role in maintaining the balance of fat metabolism in organisms, and is important for the prevention of obesity and its related metabolic syndromes. Eunkuk Park et al. showed that the combination of *Cornus officinalis* and tempeh extract

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

effectively inhibited HFD-induced weight gain in HFD-obese mice through down-regulation of the expression of adipogenic genes. (2) Salivary secretion pathway: Saliva is an important part of the oral environment, and its normal or abnormal secretion function is directly related to oral health and overall physiological homeostasis.Given that *Cornus officinalis* has the efficacy of tonifying the liver and kidneys, astringency and fixation of detachment in traditional Chinese medicine theory [17], and that the liver and kidneys are closely related to the production and distribution of fluids, we hypothesize that *Cornus officinalis* may indirectly promote salivary secretion through the regulation of the functions of related internal organs, thus maintaining oral health. (3) Cholinergic synaptic pathway: As an important information transfer mechanism in the nervous system, abnormal function of cholinergic synapses is closely related to various cognitive and behavioral disorders. It has been found that drug disruption of cholinergic activity leads to impairment of behavioral cognition, but increasing cholinergic activity improves cognition in elderly patients [18]. In particular, the cholinergic hypothesis proposed by Bartus [19] provides important theoretical support for explaining the pathogenesis of cognitive disorders such as Alzheimer's disease. The study by Yang Jige et al [20] showed that the alcoholic extract of *Cornus officinalis* significantly improved the learning and memory abilities of mice in the Aβ25-35-induced Alzheimer's disease model, which is a promising finding for the therapeutic use of *Cornus officinalis* in the treatment of cognitive disorders.finding provides experimental evidence for the potential of *Cornus officinalis* in the treatment of cognitive disorders and further supports the hypothesis that it may improve cognitive function in humans by enhancing cholinergic activity.

Based on the PPI network analysis, this study ultimately focused on three core genes - PPARG, PRKACA and NR3C1 - which occupy crucial positions in the gene network and are potentially associated with the biological effects of the active ingredients of *Cornus officinalis*. (1) The gene PPARG is a peroxisome proliferator-activated receptor, which encodes a member of the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors. PPARG is not only involved in regulating the expression of adipocyte-associated genes, which exhibit significant anti-inflammatory and anti-tumor potential, but also profoundly influences key physiological-pathological processes such as angiogenesis, apoptosis and fatty acid biosynthesis [21]. Given their multiple biological functions, PPARG and its ligands are regarded as potential chemopreventive and cancer therapeutic strategies, and such drugs have also been suggested for the treatment of diabetes and cardiovascular diseases [22]. Therefore, we hypothesized that some of the active components of *Cornus officinalis* may promote lipid metabolic homeostasis, inhibit inflammatory responses, and exhibit potential anticancer activity. (2) The PRKACA gene encodes the camp-activated catalytic subunit α of protein kinase A (PKA), which also serves as a core component of the PKA tetrameric holoenzyme, and its kinase activity plays a key role in the pathomechanisms of cardiac diseases and cancer. Studies have shown that the kinase activity of PRKACA is tightly linked to its oncogenic potential, suggesting that kinase inhibition strategies against this gene may be an effective target for the treatment of the aforementioned diseases [23]. *Cornus officinalis* has the efficacy of nourishing the heart and tranquilizing the mind in Chinese medicine theory, and we further hypothesized that its active components may play an active role in cardioprotection and anticancer. (3) The NR3C1 gene is a member of nuclear receptor subfamily group 3C1, and the glucocorticoid receptor it encodes plays a central role in transcriptional regulation, acting as a transcription factor that binds to the glucocorticoid-responsive element in the promoter of glucocorticoid-responsive genes to activate their transcription, and also acting as a regulator of other transcription factors. Notably, the NR3C1 gene has been significantly associated with a variety of psychiatric disorders, stress-related diseases and autoimmune disorders such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease and membranous nephropathy [24-25]. This reveals that *Cornus officinalis* may be able to modulate the glucocorticoid response mechanism and thus show potential efficacy in alleviating stress-related diseases and autoimmune diseases.

The present study also has some limitations: we were unable to directly detect and analyze specific metabolites in *Cornus officinalis* and failed to detect new active substances, which to some extent limits our comprehensive understanding of the medicinal potential of *Cornus officinalis*. In addition, the results of this study are all based on database prediction, and experimental validation and exploration of new active substances need to be strengthened in the future to further expand its medicinal value.

5. Conclusion

To summarize, we investigated the active components of *Cornus officinalis* by pharmacological analysis of Chinese Medicine Network. We obtained 20 active ingredients by adjusting the broad

ISSN 2706-6819 Vol.6, Issue 8: 28-43, DOI: 10.25236/IJFM.2024.060805

domains of OB and DL values through TCMSP database. Upon matching, 14 active ingredients were found to correspond to 76 host-associated target genes, respectively. Subsequently, we screened the top ten KEGG signaling pathways closely related to the 30 human target genes and found that the target genes had a key role in lipolysis. The importance of these genes was also revealed in GO enrichment analysis. Finally, using the PPI network analysis, the degree of the network nodes, we identified three core nodes, PPARG, PRKACA, and NR3C1. This reveals that *Cornus officinalis* may be able to show potential therapeutic efficacy for some disease aspects. In the future, we will also continue to conduct related studies to investigate the activity of *Cornus officinalis* in more depth.

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