Predication of the Underlying Protective Effect of Saffron on Parkinson's Disease via Network Pharmacology and Molecular Docking

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Research

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Abstract

Background

Reports indicate that saffron originated from Iran then introduced into mainland China through the earlier established trans-Tibet trade routes in ancient China. Based to the theory of traditional Chinese medicine, saffron functions by promoting blood circulation and removing blood stasis, cooling blood and detoxification, as well as relieving depression for tranquilization. Modern medical research has demonstrated several properties of saffron including antitumor, antidepressant, enhancement of immunity, cardioprotection. Notably, recent studies introduce that saffron has a neuroprotective effect, specifically in the treatment of Parkinson's disease in recent years. Nevertheless, the underlying molecular mechanism remains elusive so far. As such, this study aims to predict the underlying mechanism of saffron on Parkinson's disease through network pharmacology and molecular docking.

Methods

A functional-based network pharmacology and molecular docking model was constructed for dissecting the underlying mechanism of saffron on Parkinson's disease. Based on the Traditional Chinese Medicine System Pharmacology database (TCMSP) and the literature reviews, the putative targets of Saffron were collected. Further, the interaction networks of Drug- candidate compounds targets-Therapeutic targets-Disease were mined using the Cytoscape software. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted and the String database was used to analyze the protein interaction network. Finally, Molecular docking was used to predict the interaction between the components in Saffron and key targets by MOE 2014.09 software.

Results

A total of 9 candidate compounds from saffron corresponding to 52 therapeutic targets of Parkinson's disease were identified. The neuroprotective effect of saffron in the treatment of PD was attributed to the strong binding between crocin (including crocin I and crocin II) as well as key targets including CASP3, IL6, and MAPK8. Additionally, the auxiliary neuroprotective effects might have originated from quercetin, kaempferol, isorhamnetin, crocetin, safranal, and picrocrocin because of their slightly weaker binding capacity to the key targets.

Conclusion

Saffron exhibits a synergistic effect via multiple targets and pathways in the treatment of Parkinson's disease and our systemic pharmacological analysis provides a basis for the clinical application and in-depth study of saffron.

Introduction

Parkinson's disease (PD) is a neurological disorder characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta, with dyskinesia as the prominent clinical symptom which includes resting tremor, bradykinesia, rigidity, and postural instability [1]. The pathological mechanism of PD is complex, and multiple biological mechanisms are related to PD, including oxidative stress, immune inflammation, mitochondrial dysfunction, and accumulation of toxic proteins [2-6]. Additionally, epidemiological studies have suggested that several factors play key roles in the development of PD, including, race, age, environment, lifestyle, and heredity among others [7-10]. Notebly, a retrospective analysis of PD in 2016 revealed that the number of patients nearly tripled from 2.5 million in 1990 to 6.1 million across the globe [11]. In China, the current situation remains unoptimistic due to the surge of the aging population. Besides, a statistical analysis estimates that the Chinese PD patients will reach 4.94 million, more than half the global incidence by 2030 [12]. Dopamine-related drugs including levodopa are presently used as first-line therapy in clinical treatment based on the authoritative guideline [13, 14]. As a consequence, although it can alleviate symptoms in the short term, there is still lack of evidence-based medical evidence that the drug can reverse the pathological process of PD. The problem that has to be paid attention to in the process of clinical treatment is that patients receiving long-term treatment will develop tolerance, so it is necessary to continuously increase the dose to achieve the therapeutic effect [15-17]. The use of such drugs has caused many side effects, including dyskinesias and behavioral inhibition and control damage. Therefore, continuing to carry out in-depth research on the mechanism of PD and discovering better therapeutic drugs is the direction of future efforts [18].

Crocus sativus L., commonly known as saffron, is a perennial stemless herb of the *Iridaceae*. Notably, commercial saffron comprises the dried red stigma. Saffron is widely cultivated in Europe, Turkey, Iran, Central Asia, India, China, and Algeria, of which Iran has been the world's major producer and exporter since time immemorial (85% of the global saffron production) [19]. A lot of Islamic traditional medicine literature introduced saffron as an astringent, resolvent, and concoctive drug with therapeutic effects on gastro-hepatoprotective, urogenital disorders, antidepressants, ocular disorders, etc. [20]. The accumulating pharmacological studies of saffron indicated that it can elicit anti-tumor, anti-inflammatory, antioxidant, immunomodulatory effects, and neuroprotective action [21-25]. Notably, the pharmacological mechanism of anti-oxidation, anti-inflammatory and inhibition of toxic α-Syn protein aggregation of saffron extract may be related to ameliorating the symptoms of PD patients [26-28]. The use of new research methods to clarify the precise pharmacological mechanism of saffron has important clinical significance for further promoting the use of the drug in the treatment of PD patients. Scientists believe that the active substance groups of traditional Chinese medicine (TCM) play a therapeutic role via multi-target and multi-pathway. Of note, network pharmacology is an effective method to understand human body-drug interaction from a macro perspective, and to further explore the scientific problem on mechanism by which the complex components of TCM interfere with disease targets [29]. Given that the precise mechanism of saffron in the treatment of PD remains unclear, we sought to predict the underlying mechanism of saffron on PD through network pharmacology and molecular docking. First, we attempted to screen the related active component of saffron in therapeutic target on PD and further conducted interrelation analysis using multiple databases. Moreover, to clarify the mechanism of saffron in the

treatment of PD, the bond strength between components and disease targets were evaluated by molecular docking. The flowchart of the experimental procedures is shown in Fig. 1.

Methods

Screening the candidate compounds and targets of saffron

Conventionally, oral administration of Saffron was usually the selected route, hence absorption, distribution, metabolism, and excretion (ADME) regulated the process of drug exerting its action. Oral bioavailability (OB) and drug-likeness (DL) have been introduced to characterize the pharmacokinetic characteristics of active components, and to further provide criteria for screening active components from TCM [30, 31]. OB is a vital index to describe the relative amount of drug absorption into the blood circulation, whereas DL refers to the similarities between the active components and most currently known drugs in physical and chemical properties. Generally, compounds that meet these basic pharmacokinetic parameters might accelerate new drug discovery and improve the success rate of drug development. Based on the candidate compounds screening thresholds, this study set OB≥30% and DL≥0.18 to screen the active components of saffron using the traditional Chinese medicine system pharmacology database and analysis platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) [32, 33]. As supplementary, a literature search was conducted in PubMed (https://pubmed.ncbi.nlm.nih.gov), using the items “Parkinson”, “*Crocus sativus L*”, and “Saffron” as keywords. Based on this, we added the following active components: Crocin I, Crocin II, Safranal, and Picrocrocin. Despite these 4 components not meeting the above requirements, it was clearly mentioned highlighted in relevant literature for neuroprotective and the therapeutic effect of PD [34-37]. Further, the biological targets of the candidate compounds in Saffron were gathered from TCMSP. Moreover, α-Synuclein protein (α-Syn, protein-coding gene known as SNCA) was supplemented into the final result because of it being the main components in the Lewy body, the most important pathological markers of PD. It has been convincingly demonstrated that Crocin I and Crocin II could interact with α-Syn and inhibit the formation of aggregates *in vitro* and computer simulation studies [28, 38]. The last step was to delete duplicate targets and further ensure data quality as well as standardization using the Uniprot database (https://www.uniprot.org/).

Collection of the confirmed and potential therapeutic targets of PD

Notably, disease therapeutic targets usually include receptors, enzymes, ion channels, transporters etc. The interaction between drugs and targets triggered changes in physiological and biochemical functions of the body, to realize the regulation of drugs on the pathological and physiological state of the body and achieve the purpose of disease treatment [39, 40]. In light of all of this, the confirmation of disease targets is an important basic work in drug development and clinical diagnosis and treatment. Search terms including“Parkinson’s disease” as keywords was used to conduct a systematic data search for disease targets from databases with no date restrictions, including, Online Mendelian Inheritance in Man Databases (OMIM, http://www.omim.org/), Genecards Databases (http://www.genecards.org/, cut off value larger than 20), DrugBank Databases (https://www.drugbank.ca/), Comparative Toxicogenomics

Database (CTD, http://ctdbase.org/, Score greater than 40). This selection of databases was based on susceptibility genes, confirmed drug targets, and environmental exposure factors hinged on the focus of different databases. Further, the search results were comprehensively analyzed and converted to the corresponding official gene symbol by Uniprot Database to finally obtain the definitive targets of PD.

Construction of the drug-candidate compounds -therapeutic targets-disease network

Further interrelation analysis was performed to obtain the intersection of candidate compounds targets and therapeutic targets all the above collected, namely common targets, these candidate compounds from saffron might act directly on the therapeutic targets of PD. Then, the network of drug-candidate compounds-therapeutic targets-disease was constructed using Cytoscape software version3.7.2. Nodes with different shapes and colors represented different properties of the nodes in the visual network diagram. Specifically, the connecting line represented the subordination relationship of drug-compounds or disease-targets and the compound-targets action relationship between nodes [41].

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

In this work, we took a step further transforming the official gene symbol of common targets into entrezID using the “org.Hs.eg.db” R package of R software version 3.6.2 (x64). Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the “clusterProfiler” R package, meanwhile, associated signaling pathways were rendered using the“ Pathview” R package. The results were presented in the form of a bar chart and a bubble chart. P-value < 0.05 or Q value < 0.05 were considered statistically significant enrichment.

Construction of the protein-protein interaction (PPI) network for the therapeutic targets of PD

To predict all the potential interactions of common targets between candidate compounds targets and therapeutic targets, all the common targets were inputted into the String database (https://string-db.org/cgi/input.pl), with the species limited to “*Homo sapiens*” and “minimum required interaction score>0.4” as the basis of confidence for the interaction between proteins. Subsequently, the basic topological parameters of protein-protein interaction network including the degree value of the node (Degree), closeness centrality (CC), and betweenness centrality (BC) were calculated using the Network Analyzer tool function of String database, all the results were presented as a bar chart and 3D scatter plot.

Molecular docking simulation

The concept of molecular docking refers to placing small molecular ligands in the binding region of macromolecular receptors through computer simulation then calculating physical and chemical parameters to predict the binding affinity between them. Briefly, the X-ray crystal structure of the target protein was downloaded from the protein data bank database (PDB, http://www.rcsb.org/), and the molecular docking was performed using MOE 2014.09 software. The Triangular Matching docking

method was used to dock the molecules, then, 30 conformations of each ligand-protein complex were generated based on docking score. The docking poses between the active components and the binding pocket residues of the target protein were evaluated via visual inspection relying on the visualization function of the software. Finally, the clear images of ligand-protein interactions were retained and outputted

Results

Candidate compounds and targets of Saffron

A total of 9 candidate compounds were collected, 4 of which were collected from the primary literature as mentioned above, whereas the remaining 5 including Quercetin, Kaempferol, Isorhamnetin, Crocetin, n-Heptanal were screened from the TCMSP database. Also, the TCMSP database was used for target analysis of candidate compounds, on this basis, the repeated targets were consolidated and the above-mentioned confirmed target SNCA was added. A total of 92 targets of candidate compounds were collected and the basic pieces of information, including OB, DL, and numbers of corresponding targets are listed in Table 1.

Table 1 The list of 9 candidate compounds of Saffron and their basic pieces of information

ID	compound	OB(%)	DL	Number of target
MOL000098	Quercetin	46.43	0.28	45
MOL000422	Kaempferol	41.88	0.24	21
MOL000354	Isorhamnetin	49.60	0.31	8
MOL001406	Crocetin	35.30	0.26	7
MOL001389	n-Heptanal	79.74	0.59	2
MOL001405	Crocin I	2.54	0.12	1
MOL001407	Crocin II	1.65	0.21	1
MOL000720	Safranal	39.56	0.04	5
MOL001409	Picrocrocin	33.71	0.04	2

Potential therapeutic targets of PD

Results from four databases are merged and de-duplicated to provide a list of all confirmed and potential therapeutic targets of PD. A total of 38 therapeutic targets were screened out from Drugbank. The results were more convincing because the resources provided by Drugbank came from the confirmation information of listed drugs. Up to 104 PD related targets with cut off value larger than 20 were collected from the Genecards database while 29 therapeutic targets were collected from the OMIM database.

Notably, the Genecards database provides genes related to common human diseases, while the OMIM database focuses on the relationship between disease phenotypes and their pathogenic genes. CTD database considered the genes affected by PD related environmental toxicant exposure, and a total of 466 therapeutic targets were collected when the screening score was set as greater than 40. A total of 556 PD therapeutic targets were collected from different databases, as shown in Fig. 2. There was not much overlap between different databases, indicating that the therapeutic targets collected in this study were quite complete.

Common targets of between compounds targets of saffron and therapeutic targets of PD

Further analysis revealed that 52 common targets were observed in both candidate compounds targets of saffron and therapeutic targets of PD (Fig. 3) and their basic information regarding Uniprot ID, official gene symbol of target, and protein full name of target (Table 2). Then, this result was classified based on the main molecular function of the target proteins, and these molecular functions concentrated on apoptosis, oxidative stress, inflammation, cholinergic system disorder, and toxic protein damage. This was consistent with the recent studies on the pathological mechanism of PD, (Fig. 4).

Table 2. Basic information of common targets

Uniprot ID	Target gene	Target protein
P37840	SNCA	Alpha-synuclein
P09488	GSTM1	Glutathione S-transferase Mu 1
P05231	IL6	Interleukin-6
P15559	NQO1	NAD(P)H dehydrogenase
P09211	GSTP1	Glutathione S-transferase P
P10415	BCL2	Apoptosis regulator Bcl-2
Q16236	NFE2L2	Nuclear factor erythroid 2-related factor 2
P42574	CASP3	Caspase-3
P01100	FOS	Proto-oncogene c-Fos
P22303	ACHE	Acetylcholinesterase
P15692	VEGFA	Vascular endothelial growth factor A
Q04206	RELA	Transcription factor p65
P55211	CASP9	Caspase-9
P45983	MAPK8	Mitogen-activated protein kinase 8
P37231	PPARG	Peroxisome proliferator-activated receptor gamma
P03372	ESR1	Estrogen receptor
P24385	CCND1	G1/S-specific cyclin-D1
P49841	GSK3B	Glycogen synthase kinase-3 beta
P25963	NFKBIA	NF-kappa-B inhibitor alpha
P08684	CYP3A4	Cytochrome P450 3A4
P29474	NOS3	Nitric oxide synthase, endothelial
P09874	PARP1	Poly [ADP-ribose] polymerase 1
P04798	CYP1A1	Cytochrome P450 1A1
Q16665	HIF1A	Hypoxia-inducible factor 1-alpha
P04792	HSPB1	Heat shock protein beta-1
P05362	ICAM1	Intercellular adhesion molecule 1
P01106	MYC	Myc proto-oncogene protein
P27169	PON1	Serum paraoxonase/arylesterase 1

P10275	AR	Androgen receptor
P00533	EGFR	Epidermal growth factor receptor
P14635	CCNB1	G2/mitotic-specific cyclin-B1
P23219	PTGS1	Prostaglandin G/H synthase 1
P19320	VCAM1	Vascular cell adhesion protein 1
P17252	PRKCA	Protein kinase C alpha type
P02741	CRP	C-reactive protein
P28161	GSTM2	Glutathione S-transferase Mu 2
O15392	BIRC5	Baculoviral IAP repeat-containing protein 5
Q14790	CASP8	Caspase-8
Q03135	CAV1	Caveolin-1
P16435	POR	NADPH–cytochrome P450 reductase
P08172	CHRM2	Muscarinic acetylcholine receptor M2
Q13085	ACACA	Acetyl-CoA carboxylase 1
P04049	RAF1	RAF proto-oncogene serine/threonine-protein kinase
P07339	CTSD	Cathepsin D
Q16678	CYP1B1	Cytochrome P450 1B1
P14867	GABRA1	Gamma-aminobutyric acid receptor subunit alpha-1
P35869	AHR	Aryl hydrocarbon receptor
P06400	RB1	Retinoblastoma-associated protein
P11229	CHRM1	Muscarinic acetylcholine receptor M1
P19419	ELK1	ETS domain-containing protein Elk-1
P35348	ADRA1A	Alpha-1A adrenergic receptor
P20309	CHRM3	Muscarinic acetylcholine receptor M3

Construction of the drug- candidate compounds-therapeutic targets-disease network

The network of the drug, candidate compounds, therapeutic targets, and PD was built to analyze the complex interactions between saffron and PD. As shown in Fig.5, the network comprised 153 edges and 63 nodes including 52 therapeutic targets of PD represented by green circular nodes, and 9 compounds of saffron represented by orange triangle nodes. The node size and depth of the color determined the degree value of the node, for further explanation, the larger the size and the darker the color, the greater

the degree value of the node. The current findings confirm that the relationship between candidate components and therapeutic targets is not a simple rule like one-to-one correspondence, but complex, for instance, one-to-many or many-to-one. Many candidate components were related to multiple therapeutic targets of PD, particularly quercetin and kaempferol, which might be the important compounds in the treatment of PD.

GO and KEGG enrichment analyses

The purpose of GO enrichment was to clarify the potential mechanism of saffron in the treatment of PD from the biological process (BP), cellular component (CC), molecular function (MF), respectively. The results of GO enrichment of 52 common targets were depicted as a representative bar chart, the longer the bar, the smaller the p-value is, namely, the more significant the enrichment is. As shown in Fig. 6, the top 6 biological processes implicated in the treatment of PD were cellular response to chemical stress, response to the xenobiotic stimulus, cellular response to the xenobiotic stimulus, response to the metal ion, response to oxidative stress, and cellular response to oxidative stress. In terms of cellular components, the top 6 were membrane raft, membrane microdomain, membrane region, transcription regulator complex, an integral component of postsynaptic membrane, and axon terminus. Additionally, DNA-bind transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, activating transcription factor binding, ubiquitin-like protein ligase binding, and glutathione binding were the top 6 molecular functions implicated in the treatment of PD. Fig. 7 is a simplified bubble chart that describes the results of KEGG enrichment, as its purpose was to clarify the top 16 signal pathways for the treatment of PD using saffron. In general, PD-related pathways including PI3K/Akt signaling pathway, apoptosis signaling pathway, and TNF signaling pathway showed significant enrichment. For clearer presentation, the specific position and function of common targets are colored red in the signaling pathway in Fig .8.

Protein-protein interaction (PPI) network for the therapeutic targets of PD

In this work, the protein-protein interaction (PPI) complexity of 52 common targets is clearly shown in Fig. 9 (A), Further, the degree values of the top 30 proteins in interaction relationship are shown in Fig. 9 (B). Here, the degree value of protein was a critical parameter, a direct indication of the importance in the network, and the protein with high a degree value was more likely to be the core target in the treatment of PD. To describe the topological properties of the nodes and comprehensively evaluate the status of proteins in the PPI network, a three-dimensional scatter plot was used to show the degree value, closeness centrality (CC), and betweenness centrality (BC) on each node (Fig. 9) (C). There was a significant positive correlation between them. Generally, the closeness centrality and betweenness centrality of protein with high degree value was also greater, indicating that the protein directly interacts with many proteins and act as a "bridge" to mediate the indirect interaction between other proteins. This conclusion implies that CASP3, IL6, MAPK8 were the three most important genes in the PPI network, which might be the core proteins of PD.

Molecular docking

Multiple studies have reported that the active components of saffron, including quercetin, kaempferol, isorhamnetin, crocetin, crocin I, crocin II, safranal, and picrocrocin exhibit anti-apoptotic and anti-inflammatory properties [42-48]. The above results suggest that CASP3, IL6, and MAPK8 are at the core of the PPI network, consistent with the literature, there were closely related to neuronal apoptosis and neuroinflammation of PD [49-51]. Therefore, additional extensive research is essential to explore the effect of saffron on the therapeutic targets of PD. The molecular docking technology used in this work can be presented as macro, micro, and local state of the interaction between compounds and core proteins. The crystal structure of CASP3 (PDB: 5IC4), IL6 (PDB: 1N26), and MAPK8 (PDB: 4QTD) were retrieved from the PDB database and executed protonated and energy minimized before molecular docking. The docking score of the 8 compounds and 3 proteins was shown by the heatmap (Fig. 10). The scores were used to describe the binding free energy between the compounds and the proteins, i.e, the smaller the value, the lower the binding free energy, indicating a more stable binding between compounds and the proteins. Furthermore, it was observed that the binding free energy of crocin I and crocin II was less than -8.0 kJ/mol. It was also suggested that crocin I and crocin II selectively act on the targets associated with apoptosis and inflammation, which then functioned as the limitation of inflammation and apoptosis to play a neuroprotective role.

Discussion

Being a complex disease affecting the central nervous system, PD has become a major threat to human health, and, researchers indicate that the situation is getting worse in the years ahead. The apparent pathological mechanism is a decrease of dopaminergic neurons in the substantia nigra pars compacta (SNpc), causing the depletion of dopaminergic neurotransmitters in the striatal projection neurons, and eventually leading to the functional imbalance between the dopaminergic system of substantia nigra and the cholinergic system of the striatum [52]. The death of dopaminergic neurons involves complex pathogenic factors and pathological mechanisms, suggesting that it is difficult for drugs with a single target to achieve a satisfactory therapeutic effect in the treatment of PD. Traditional TCM has a long application history in China and other Asian countries, besides, TCM is considered the pioneer of “multi-component and multi-target pharmacology” [53]. Based on the theory of TCM, PD belongs to the category of tremble syndrome which considers deficiency of body caused by old age, liver and kidney deficiency, and wind evil stirring internally caused by tendons dystrophy as its etiology. According to the theory of syndrome differentiation and treatment (“bian zheng shi zhi” in Chinese) in traditional Chinese medicine, the treatment principle of PD is to tonify liver and kidney, balance the liver and extinguish wind, nourish blood and soften tendons. Notably, this work was intrigued by a classical famous formula, Five Dragon Tremor Decoction (“wu long zhen chan tang” in Chinese) that was first documented in the classical Chinese medical book Discussion on Febrile Disease (“wen bing Tiao bian” in Chinese) in the Qing Dynasty (18th century). Saffron is an important adjuvant in this formula and plays a significant role in promoting blood circulation and removing blood stasis. Further, it has been suggested that saffron might be a potential drug for the treatment of PD, a scenario that warrants in-depth investigation. Therefore, TMC has its understanding and characteristics on the pathological mechanism and treatment of PD.

Summarily, it executes a multi-component, multi-target, and multi-signaling pathways to achieve the purpose of disease treatment. Here, we used the network pharmacology method to clarify how the components of saffron interfere with targets and related pathways of PD disease.

Recent research findings reveal that saffron extract harbors an antidepressant effect by inhibiting central inflammation, ameliorating oxidative stress, increasing the expression of synaptic plasticity-related proteins in hippocampal neurons, and regulating intestinal microflora [54-56]. Additionally, our findings show that crocin reduced the structural damage in soma volume and axon length of neurons and inhibited their spontaneous discharge in dopaminergic neurons in the ventral tegmental area (VTA) [57]. Moreover, evidence from clinical trials confirms the antidepressant efficacy of saffron extract comparable to Citalopram [58]. The above research results inspire the exploration of whether saffron extract protects the substantia nigra pars compacta, which is close to the space of VTA and dominated by dopaminergic neurons. So far, no clinical trial data supports the idea that saffron is a potential drug in preventing and treating PD, however, the biological activity of neuroprotective is gradually being clarified *in vitro* studies. In an animal model of PD induced by rotenone, an inhibitor of the Mitochondrial Electron Transport Chain (ETC), crocin activated the PI3K/Akt pathway, then, phosphorylated Akt inhibited the phosphorylation of GSK-3β to reduce the phosphorylation of α-Syn and eventually inhibited the aggregation of α-Syn [59]. Importantly, phosphorylated α-Syn is more aggressive and more toxic compared to its prototypes [60]. Also, the activation of mTOR by p-Akt promoted the expression of downstream effector p70S6K and increased the level of Bcl-2 in preventing cell apoptosis. Also, p-Akt inhibited the expression of Caspase-9 by inhibiting the transcription of FoxO3a to prevent the initiation of the apoptosis cascade reaction [59]. Therefore, the activation of the PI3K/Akt pathway is an effective intervention approach in reducing the abnormal aggregation of toxic proteins and preventing cell apoptosis. PI3K/Akt signal transduction pathway was significantly enriched in our study, and this was consistent with a growing body of evidence in the literature.

Additional studies have recently suggested central inflammation as one of the key factors causing the death of dopaminergic neurons. In recent years, there has been an increasing focus on PD-related neuroinflammation caused by the aggregation of α-Syn and microglial activation caused by intestinal flora imbalance [61]. Also, crocin and crocetin might reduce the production of TNF-α and IL-1β induced by LPS in the rat brain, as well as simultaneously inhibit the activation of NF-κB signal pathway induced by LPS, with the ultimate goal of inhibiting the inflammatory factors released by activated microglia [62]. Another study also confirmed that crocin significantly reduced the levels of TNF-α and lipid peroxidation in the brain of PD model animals induced by 6-OHDA, and improved motor ability and cognitive function [63]. Noteworthy, reports indicate that the crocus formula ameliorates the blood glucose level of diabetic rats by regulating intestinal flora [64]. In summary, the anti PD effect of saffron might involve the inflammatory reaction mediated by microglia, inhibit the aggregation of α-Syn and regulate the intestinal flora in exerting a regulatory effect on central inflammation.

In our study, a series of network pharmacology methods including the OB and DL evaluation, target prediction, pathway identification, PPI network establishment, and molecular docking technology were

applied to clarify the underlying mechanism of saffron in the treatment of PD. Our analysis provides convincing evidence that CASP3, IL6, and MAPK8 are the key targets to expend in the treatment of PD with saffron. Notably, crocin I and crocin II demonstrated strong binding to CASP3, IL6, and MAPK8, the binding free energy close to or even less than-8.0 kJ/mol, significantly better compared to others. Besides, the auxiliary neuroprotective effects potentially originated from quercetin, kaempferol, isorhamnetin, crocetin, safranal, and microglial due to their binding capacity to CASP3, IL6, and MAPK8. Our previous studies revealed that Saffron comprised flavonoids and carotenoids, especially rich in crocin I and crocin II [65]. Moreover, pharmacokinetic studies indicated that crocins were absorbed by passive transcellular diffusion to a high extent within a short time interval over the intestinal barrier, then, penetrated in a quite slow process in the blood-brain barrier to reach the central nervous system [66]. All the evidence provided in these studies confirms the conclusion that saffron exhibits a satisfactory development prospect because of the potential neuroprotective effects in the treatment of PD. It is worth noting that, molecular docking is only a method to simulate the binding mode and affinity of active components and biological macromolecules by computer software from some algorithms. In fact, the interaction mode of the two molecules in vivo is very complex and affected by many physiological factors. Future research needs to verify the current results by designing in vitro and in vivo experiments, and provide important information for PD drug development.

Conclusion

In summary, we used a network pharmacology strategy to predicate the compounds with biological activity from saffron and the possible targets and pathways related to PD. Consequently, there was sufficient evidence that crocin I and crocin II play a neuroprotective role by interfering with the target of apoptosis and inflammation pathway, and the good drug-target relationship implies that saffron extract may have potential research and development value in the treatment of PD, namely MAPK8, CASP3 and IL6, which have been predicted as the hopeful target of saffron on PD. The network pharmacology was an effective tool to explore the material basis of TCM and comprehensively reveal the effects of multi-component, multi-target, and multi-path on the treatment of diseases. However, further experimental verification is essential, an important next step to consider.

Abbreviations

PD: Parkinson's disease; SNpc:substantia nigra pars compacta; VTA: ventral tegmental area; TCM: Traditional Chinese Medicine; OB: oral bioavailability; DL: drug likeness; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein–protein interaction CC: closeness centrality; BC: betweenness centrality; α-Syn: alpha-synuclein; CASP3: caspase-3; IL6: interleukin-6; MAPK8: mitogen activated protein kinase-8.

Declarations

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Authors' contributions

WP conceived and designed the research methods. G-JJ performed the network pharmacology analysis and molecular docking verification. Bathaie SZ, J-GJ revised the manuscript. G-JJ,X-Zj, H-SS, C-BQ, Z-SC wrote the paper. All authors were responsible for reviewing data. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Figures

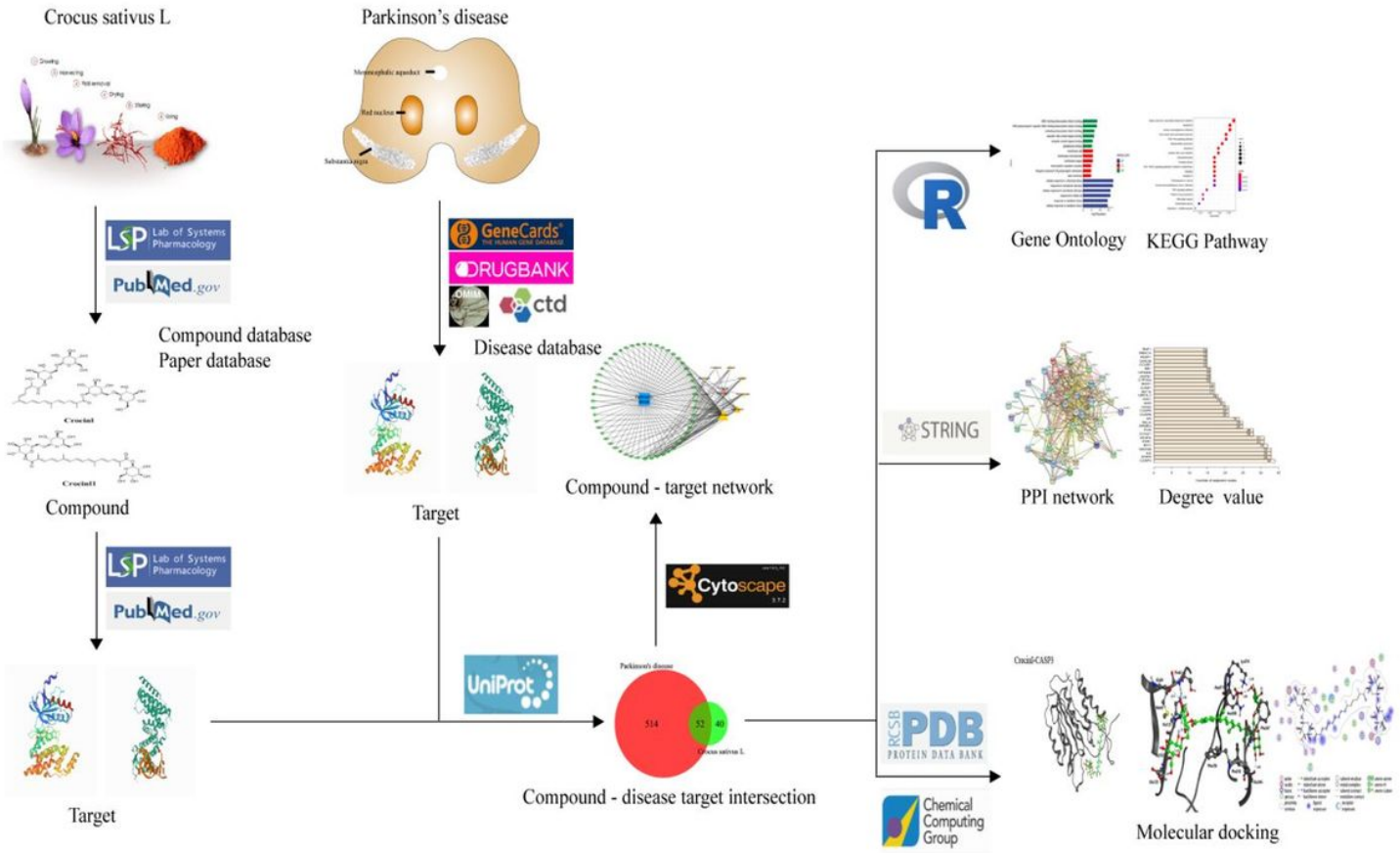

Figure 1

The flowchart of the network pharmacology-based strategy for predicting the mechanisms of saffron on PD

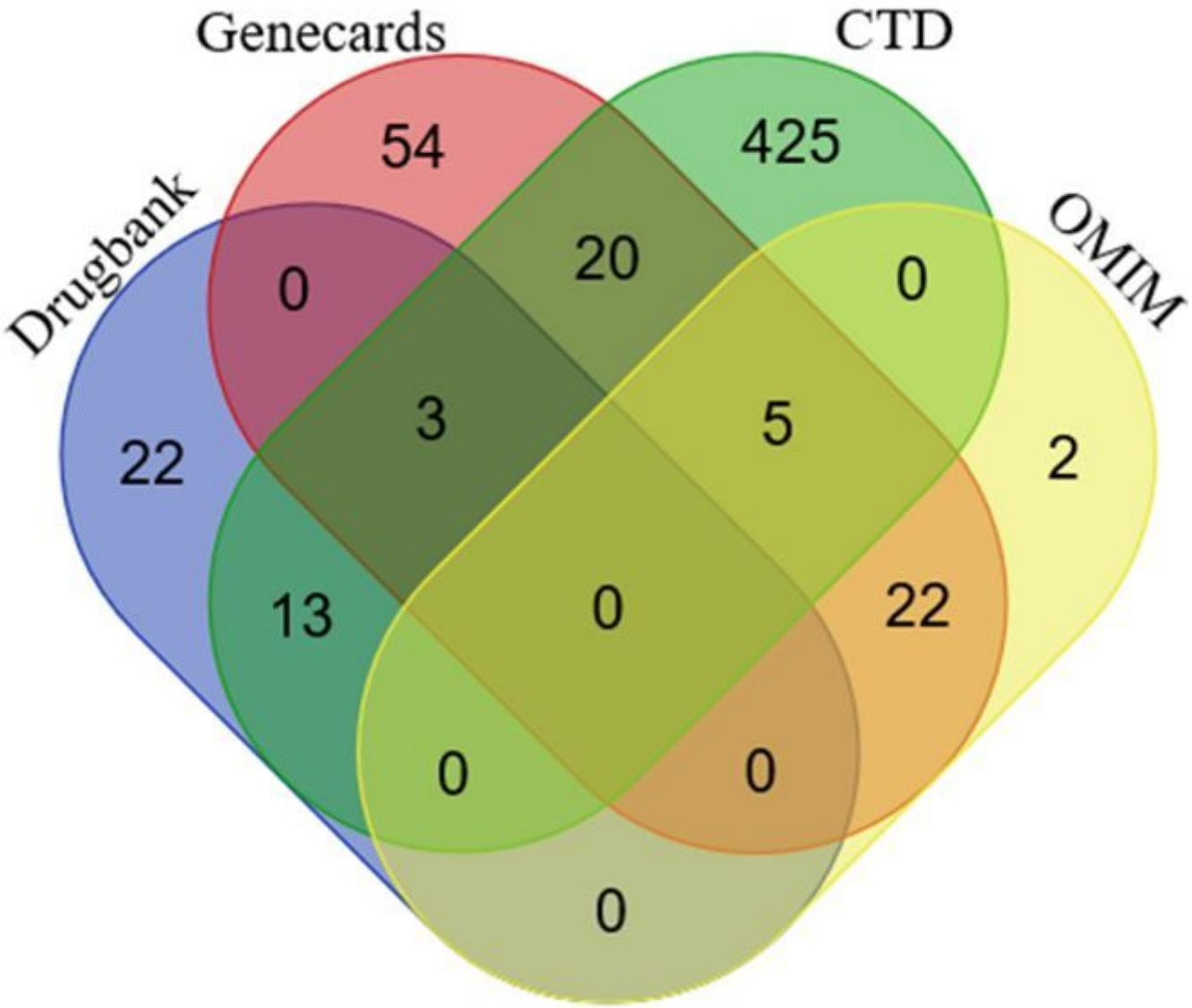

Figure 2

The number of therapeutic targets of PD collected in different databases. After merged and de-duplicated, a total of 556 compounds were obtained from 4 databases, including 38 from Drugbank, 104 from Genecards, 466 from CTD and 29 from OMIM.

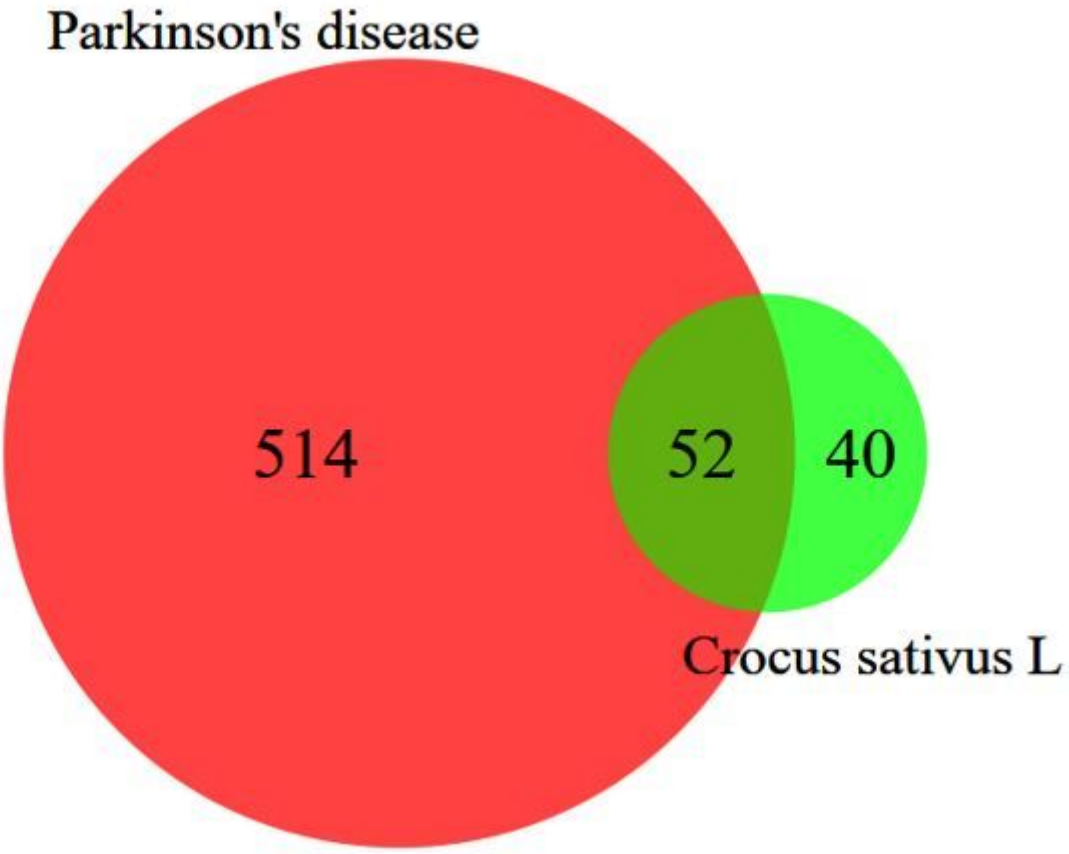

Figure 3

Common targets between candidate compounds targets of saffron and therapeutic targets of PD. 52 common targets were observed in both candidate compounds targets of saffron and therapeutic targets of PD.

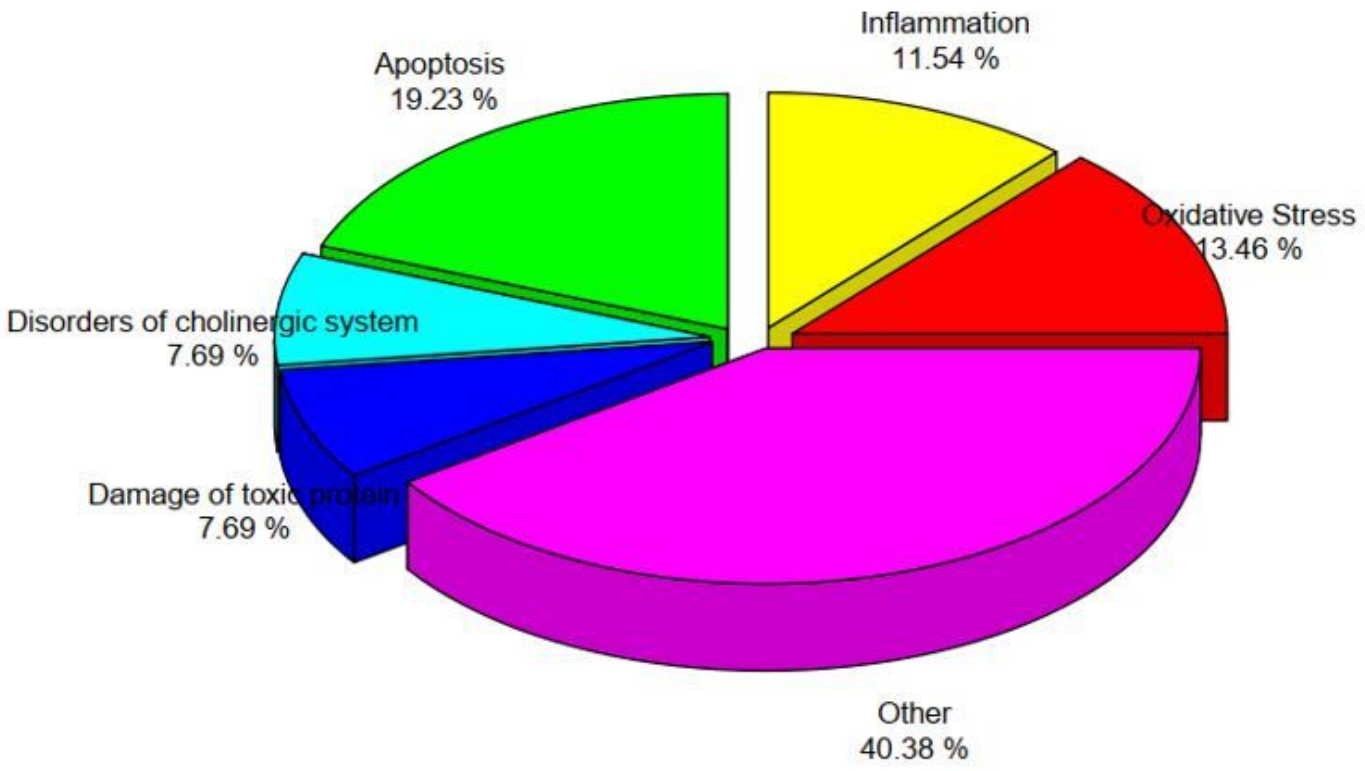

Figure 4

Functional classification of target protein. The largest proportion is apoptosis (19.23%), followed by oxidative stress(13.46), inflammation(11.54%), cholinergic system disorder(7.69%), and toxic protein damage(7.69%).

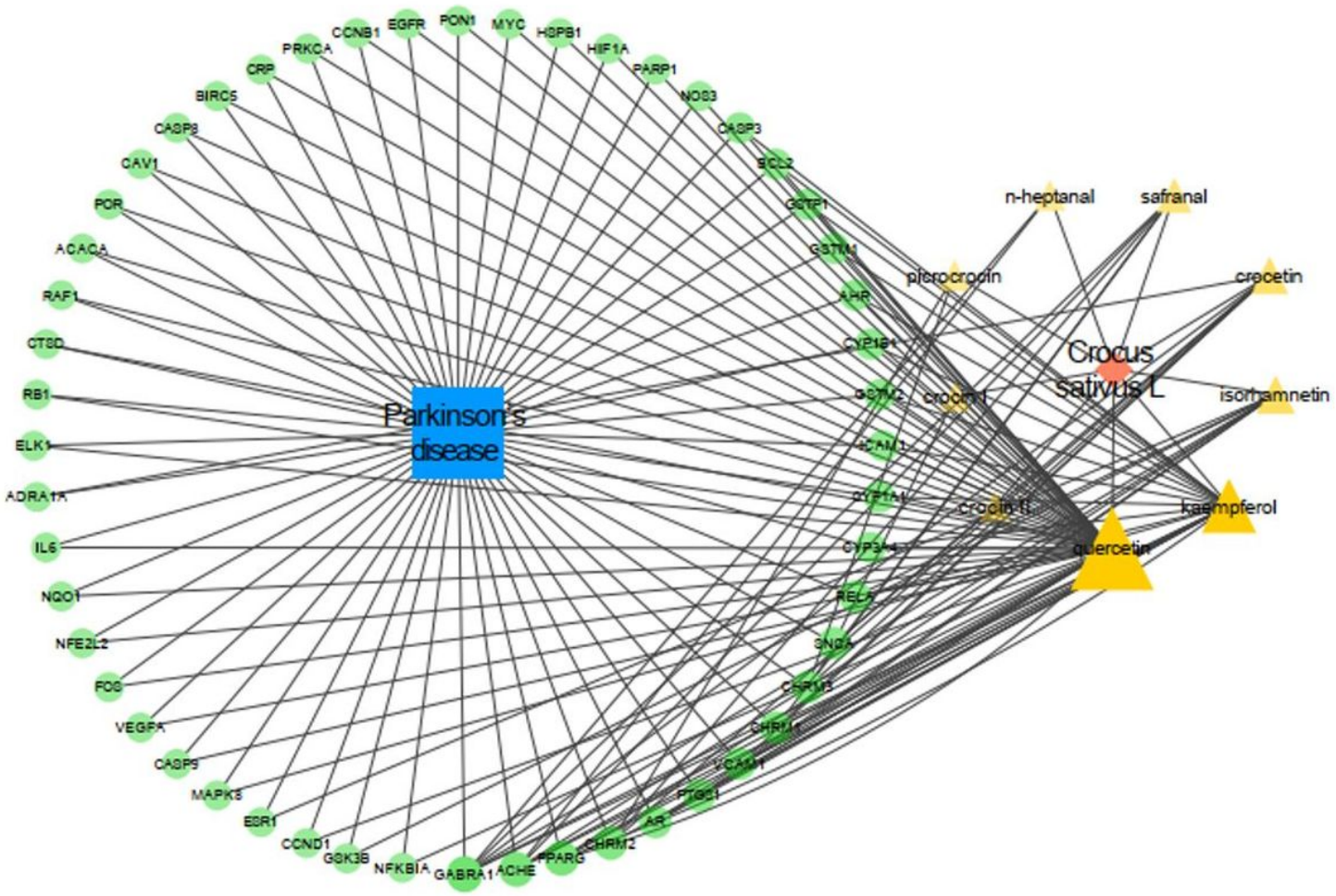

Figure 5

The drug-candidate compounds targets-therapeutic targets-disease network. The network comprised 153 edges and 63 nodes including 52 therapeutic targets of PD represented by green circular nodes, and 9 compounds of saffron represented by orange triangle nodes.

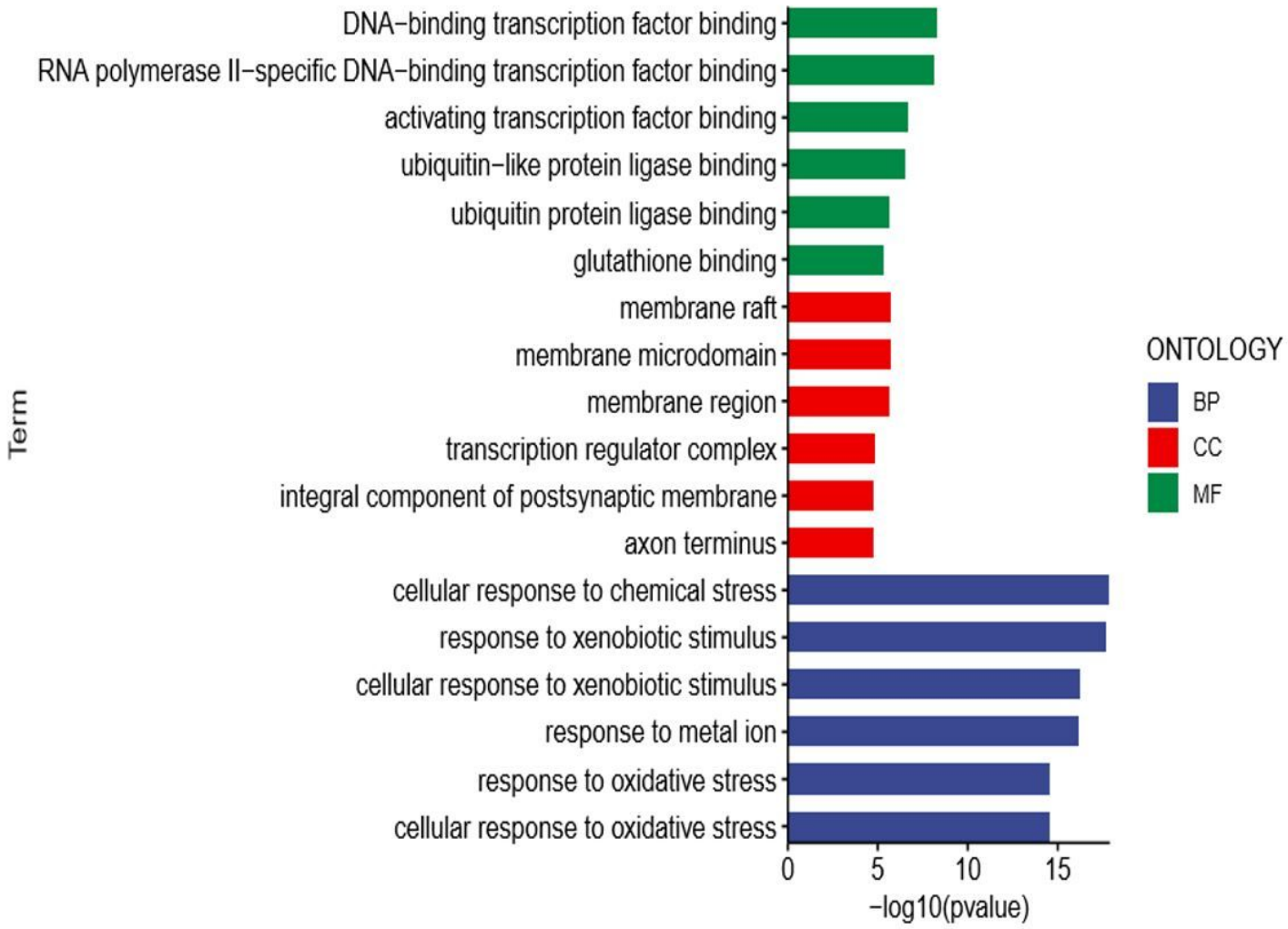

Figure 6

GO enrichment analysis of 52 common targets. The top 6 biological processes implicated in the treatment of PD were indicated in blue, cellular component were indicated in red, and molecular function were indicated in green.

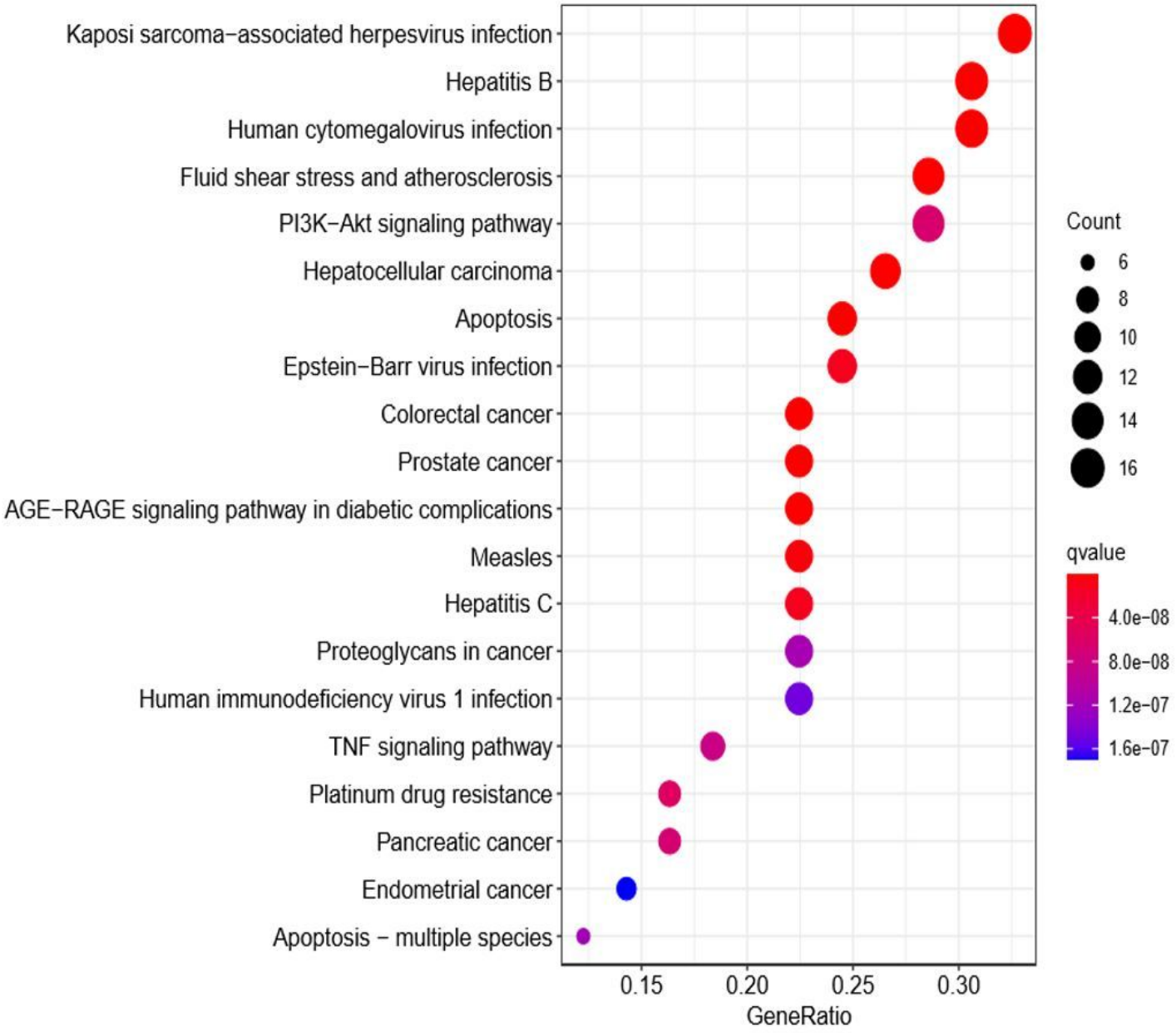

Figure 7

KEGG enrichment analysis of 52 common targets. The color of the bubbles changed from blue to red, indicating that the statistical significance was gradually enhanced and the size of the circle indicated the number of genes enriched in the pathway-the larger the circle, the more genes were enriched.

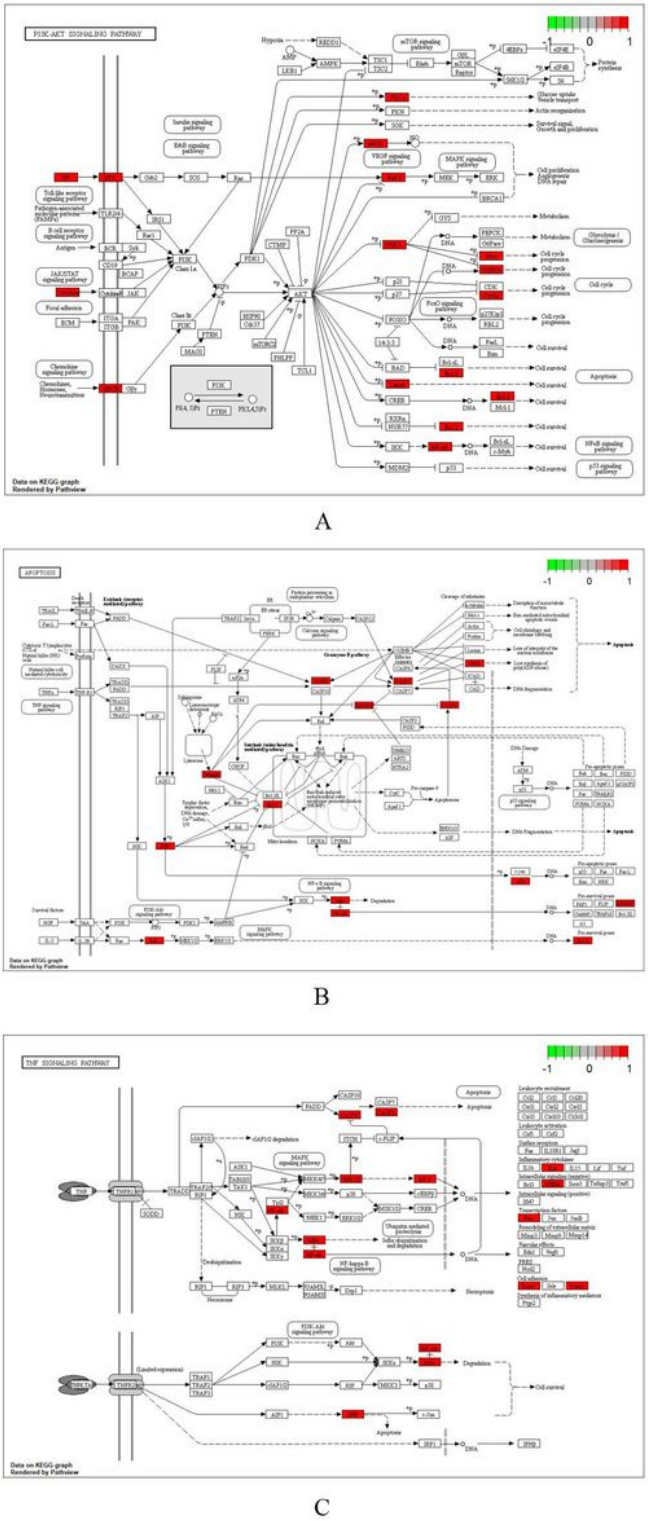

Figure 8

The specific position and function of common targets in the signaling pathway; (A) PI3K/Akt signaling pathway; (B) apoptosis signaling pathway; (C) TNF signaling pathway.

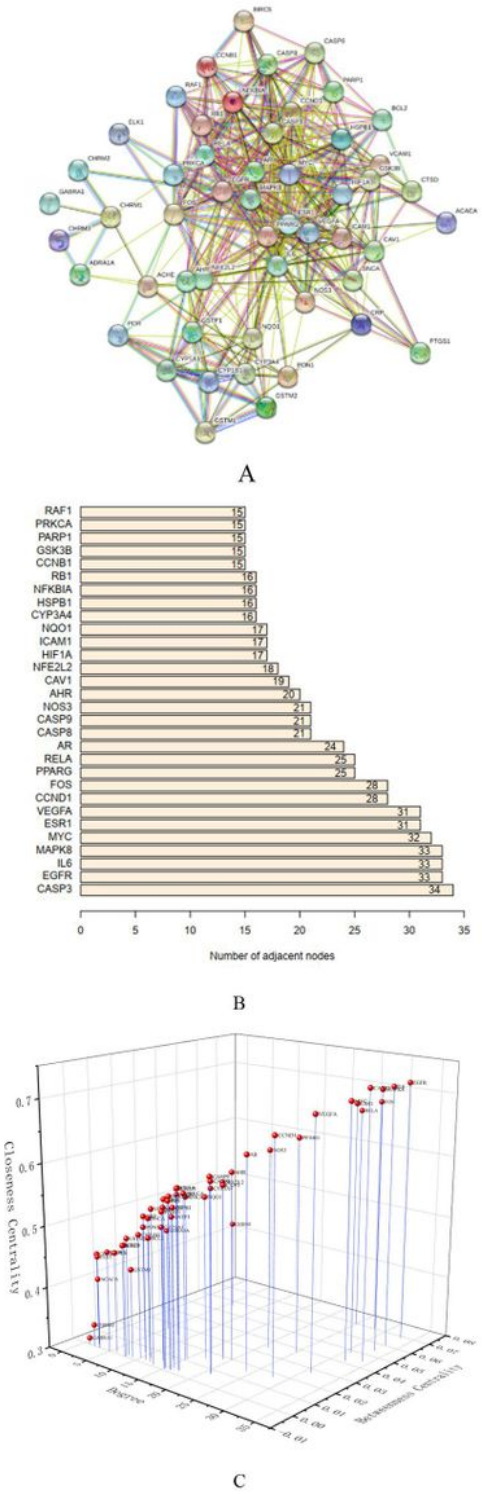

Figure 9

Protein-protein interaction (PPI) network; (A) PPI in the three-dimensional chart; (B) degree values of each protein; (C) 3D scatter plot of degree value, closeness centrality and betweenness centrality.

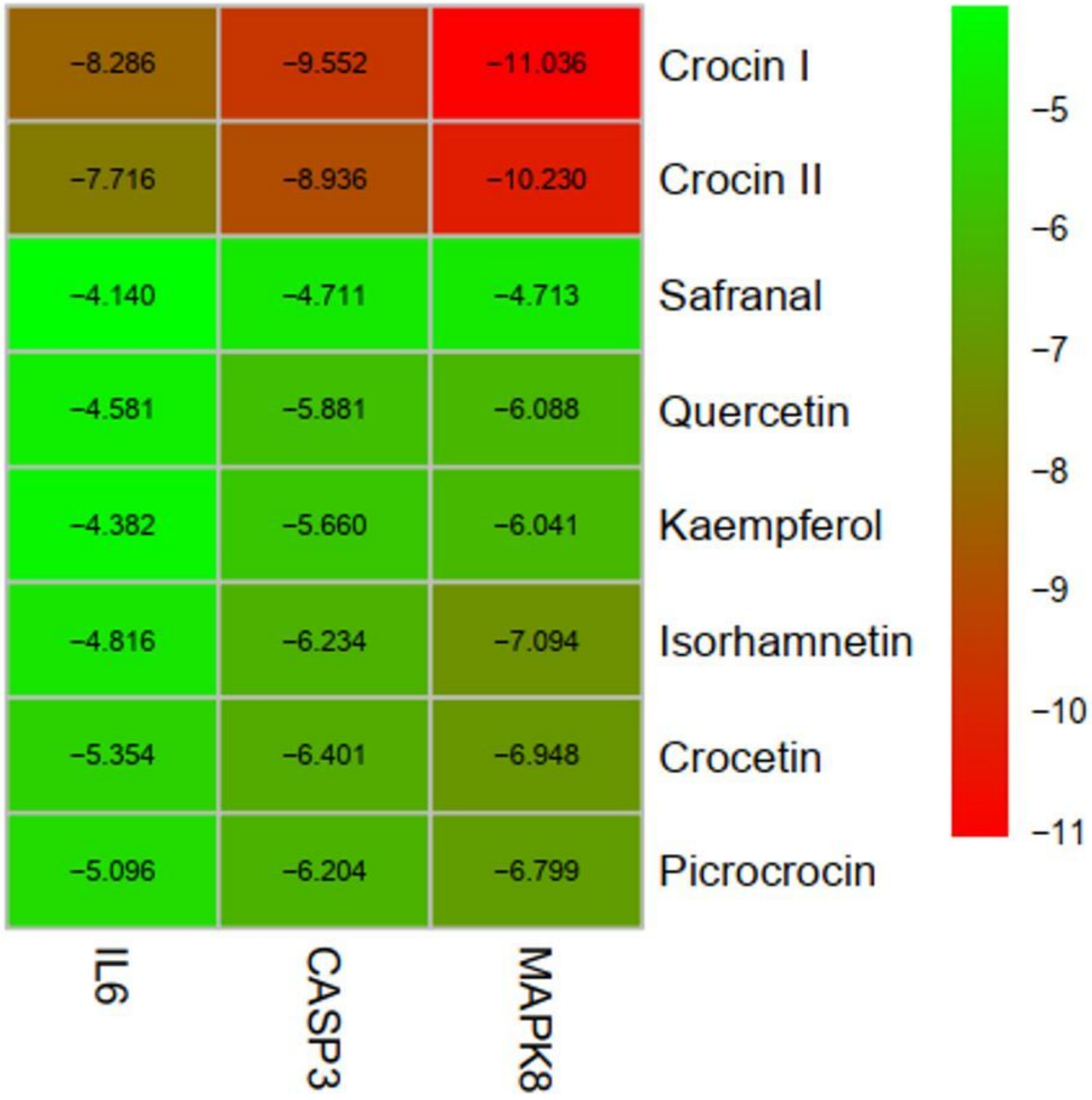

Figure 10

The heatmap of docking score. The redder the heatmap color is, the smaller the docking score is, the stronger the binding ability between compounds and the proteins.

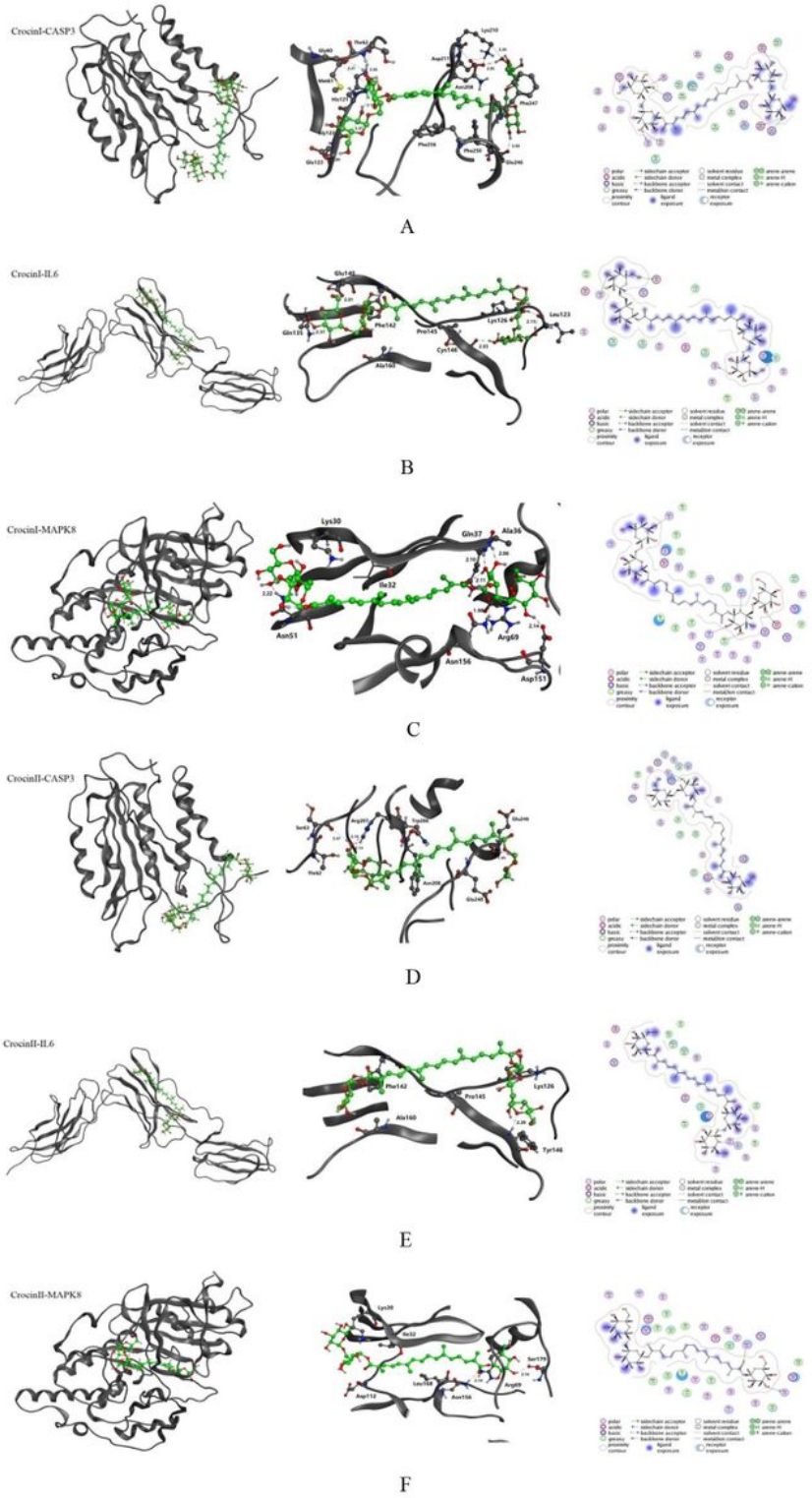

Figure 11

Dominant conformation of molecular docking between compounds and target proteins. The molecular docking was performed by the interaction between the hydrogen bonds interactions of crocin and amino acid residues of MAPK8, CASP3 and IL6, moreover, the binding strength of crocin I to target protein was stronger than that of crocin II. Specifically, the docking sites of crocin I with MAPK8 including Asn51, Gln37, Ala36, Asn156, and Asp151, and the docking sites with IL6 including Cys146, Leu123, Gln135, and

Glu140. Crocin I binds with the two peptide chains of CASP3 and forms the largest number of hydrogen bonds interactions. This makes its binding strength stronger than IL6 but less than MAPK8.