



## The mechanism of *Callistephus chinensis* flavonoid compounds in the treatment of diabetes using network pharmacology and molecular docking

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**Abstract** The purpose of this project is used for exploring the mechanism of *Callistephus chinensis* in the treatment of diabetes by network pharmacology and molecular docking methods. The target of *Callistephus chinensis* was obtained from SwissTargetPrediction database, while the target related to diabetes was obtained from GeneCards and OMIM databases. The target was added in String database to build the protein interaction network. GO biological process enrichment analysis and KEGG pathway enrichment analysis were carried out by Metascape software, then the target-pathway network was constructed. Molecular docking was carried out in Discovery Studio 2016 Client software to verify the binding force of *Callistephus chinensis* flavonoid compounds with key targets. In this study, 10 potential active components were selected from the flavonoid monomer compounds of *Callistephus chinensis*. 1 847 biological processes (BP), 126 cell compositions (CC) and 256 molecular functions (MF) were obtained by GO enrichment analysis; a total of 194 pathways were involved in KEGG enrichment analysis of 192 cross targets. Network analysis showed that quercetin was the main active component of flavonoids in the treatment of diabetes, AKT1, TNF, VEGFA, EGFR, SRC and other related signals were in relation to the treatment of diabetes. This study showed that *Callistephus chinensis* flavonoid compounds play a role in the treatment of diabetes by regulating multi-target and multi-pathway.

**Keywords:** *Callistephus chinensis*; flavonoid compounds; diabetes; network pharmacology; molecular docking

### 1 Introduction

The incidence of diabetes has risen dramatically worldwide in recent years <sup>[1]</sup>. Diabetes is a chronic disease caused primarily by the body's insufficient or excessive production of insulin <sup>[2]</sup>. Numerous studies have shown that diabetics in a state of prolonged hyperglycaemia can cause a series of diabetic complications, including diabetic nephropathy, diabetic cardiovascular complications and hepatic complications. It's a major factor in the death of

people with diabetes <sup>[3]</sup>. Current drugs used to treat diabetes, such as acarbose and miglitol, often cause some serious side effects with prolonged use, particularly diarrhoea and associated bowel pain <sup>[4]</sup>. Some active ingredients can be obtained by isolating different species of natural plants, such as flavonoids from *Callistephus chinensis* <sup>[5]</sup>, and natural medicines are potential sources of hypoglycaemic agents due to their low toxicity, high efficacy and few side effects <sup>[6]</sup>. These active ingredients inhibit the active product of  $\alpha$ -glucosidase, thereby lowering blood sugar and preventing diabetes and its complications. Therefore, plant-derived drugs provide a new approach for the

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treatment of diabetes.

*Callistephus chinensis* is a newly cultivated variety of Asteraceae, and its main bioactive components include flavonoids, terpenoids, inulin, etc. It has anti-diabetes, anti-oxidation and antibacterial effects, and has been widely cultivated and has good development prospects [7]. Flavonoids is one of the main active ingredients in *Callistephus chinensis*, is highly active potential sources of  $\alpha$ -glycosidase inhibitor has the characteristics of the treatment of diabetes, but its mechanism is unclear [8-10].

Based on the study of flavonoids monomer compounds as the core of *Callistephus chinensis*, follow the evaluation method of network pharmacology guide. From the perspective of network pharmacology, the mechanism of action of flavonoids in the treatment of diabetes mellitus was predicted, indicating that flavonoids have multi-component, multi-target and multi-pathway properties for the treatment of diabetes mellitus, which lays the foundation for further study of its molecular mechanism.

## 2 Experimental method

### 2.1 Network pharmacology research

#### 2.1.1 Screening of *Callistephus chinensis* active ingredients and targets

There are nineteen flavonoid monomers in *Callistephus chinensis*, based on previous research by our group. The nineteen compounds are as follows: Apigenin-7-*O*- $\beta$ -D-glucoside, kaempferol-7-*O*- $\beta$ -D-glucoside, apigenin-7-*O*- $\beta$ -D-glucuronidemethylester, apigenin-7-*O*- $\beta$ -D-(6''-acetyl)-glucoside, apigenin-7-*O*- $\beta$ -D-ethylester, apigenin, quercetin, kaempferol, luteolin, myricetin, hyperin, afzelin, quercitrin, rutin, naringenin, eriodictyol, (2S)-homoeriodictyol-7-*O*- $\beta$ -D-glucopyranoside, (E)-4',4,10-trihydroxy-siamaurone, (Z)-4',4,10-trihydroxy-siamaurone. From SwissADME database (<http://www.swissadme.ch/>), suitable candidate active ingredients were selected with pharmacokinetic of “High” and druglikeness of “Yes” as screening conditions. In

the SwissTargetPrediction database (<http://www.swisstargetprediction.ch/>) “human” is selected as a species, the *Callistephus chinensis* for screening for active ingredient of targets, which are expressed by abbreviation.

#### 2.1.2 Diabetes target acquisition

In the GeneCards database (<https://www.genecards.org/>) and the OMIM database (<https://omim.org/>), “diabetes” is set as the keyword to search for related target. After the name of the target is obtained, Excel spreadsheets are established to eliminate repetition, and it is used to build a library of disease targets.

#### 2.1.3 Key target acquisition

In the Venny 2.1 online platform (<https://www.liuxiaoyuyuan.cn>) the *Callistephus chinensis* compound targets and disease targets are entered for Venn mapping and the intersection of the resulting active ingredient targets and disease targets are the key targets.

#### 2.1.4 Protein interaction network diagram establishment

The common targets obtained from the analysis were imported into the String database (<https://string-db.org/>) to construct a target network map with “human” as the species, and the constructed PPI was imported into the software Cytoscape 3.9.0 software to screen the *Callistephus chinensis* core targets.

#### 2.1.5 GO bioprocess enrichment analysis and KEGG pathway enrichment

To clarify the role of target proteins interacting with target genes in gene function and signaling pathway, GO bioprocess enrichment analysis and KEGG pathway enrichment analysis were performed at the Metascape platform.

### 2.1.6 “Disease-pathway-target-drug” network diagram established

The “disease-pathway-target-drug” relationship network diagram was obtained by Cytoscape software, where the nodes represent diseases, compounds, targets, and pathways.

### 2.2 Core target crystal structure processing and molecular docking

Target 3D structures were searched and downloaded from the PDB database (<https://www.rcsb.org>). The water molecule and original ligand of the target protein were removed and hydrotreated by Discovery Studio 2016 Client. Finally, the Discovery Studio 2016 Client software was run for molecular docking, and the LibDock score was used to evaluate

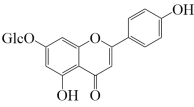
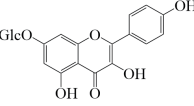
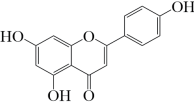
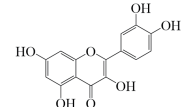
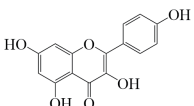
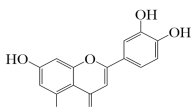
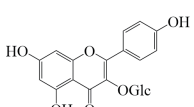
the matching ability of compounds and receptors.

## 3 Results and analysis

### 3.1 Database of active constituents and targets of *chrysanthemum*

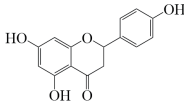
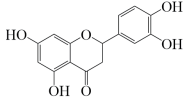
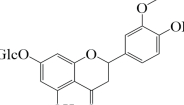
After screening and target prediction, 10 active ingredients were obtained. These are listed in Table 1. Using “diabetes” as the keyword in the GeneCards database and the OMIM database, the target names were obtained and a form table was established to eliminate the duplicated targets, and 6 486 related targets were obtained. A total of 192 targets were obtained by intersecting with the targets of flavonoids isolated from 247 *Callistephus chinensis*. Finally, Venn diagram was created using Venny 2.1, an online platform. Fig. 1 shows the result of the Venn diagram.

**Table 1** The potential active ingredient of the *Callistephus chinensis*

No.	Name	Structure
1	Apigenin-7-O-β-D-glucoside	
2	Kaempferol-7-O-β-D-glucoside	
3	Apigenin	
4	Quercetin	
5	Kaempferol	
6	Luteolin	
7	Afzelin	

(to be continued)

Continued Table 1

No.	Name	Structure
8	Naringenin	
9	Eriodictyol	
10	(2S)-homoeriodictyol-7-O-β-D-glucopyranoside	

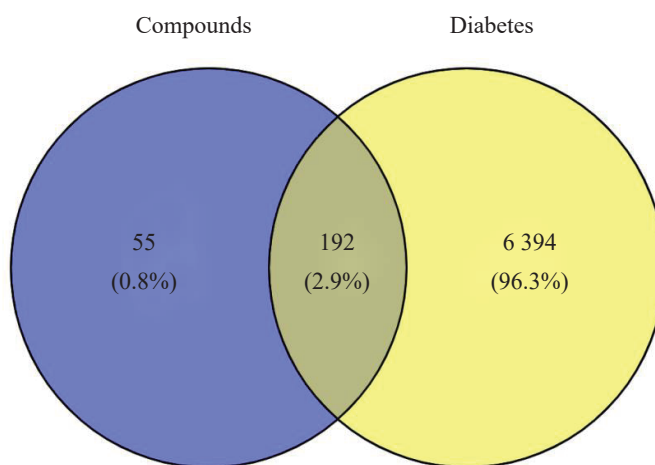


Fig. 1 The Venn diagram of *Callistephus chinensis* and disease targets

### 3.2 Protein interaction network (PPI) results

The 192 targets were entered into the String database to obtain the PPI network of the relevant diabetic targets of the flavonoid monomer compounds isolated from *Callistephus chinensis*. The higher the number of target interaction nodes, the higher the value of degrees of freedom, and the more critical the core position in this network, as shown in Fig. 2. According to the degree value, the top 10 targets are AKT1, TNF, VEGFA, EGFG, SRC, ESR1, HIF1A, HRAS, PTGS2 and MTOR. Among them, AKT1, TNF, VEGGFA, EGFG and SRC Degree values were high, indicating that the above targets may be the core targets of *Callistephus chinensis* for the disease, which are important for the therapeutic effect.

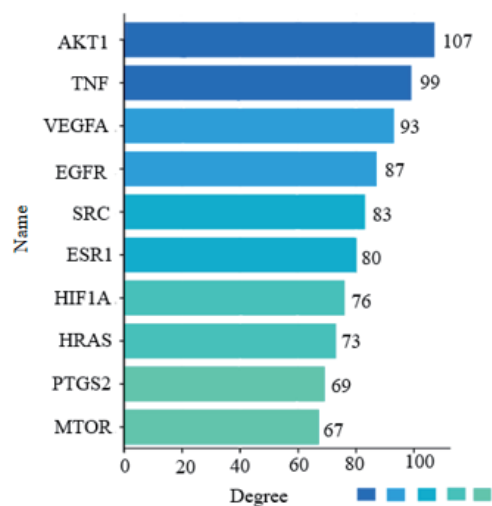


Fig. 2 PPI network bar graph of flavonoid monomer compounds isolated from *Callistephus chinensis* for the treatment of diabetes mellitus

### 3.3 GO bioprocess enrichment, KEGG pathway enrichment and construction of target-pathway network

To gain a better understanding of the identified target genes, GO enrichment analysis and KEGG enrichment analysis were performed using the Metascape platform. For the GO enrichment analysis, the top 10 results were visualized as in Fig. 3, with 1 847 biological processes (BP), 126 cellular compositions (CC) and a total of 256 molecular functions (MF). The results showed relevance to biological processes such as circulating nucleotide mediated and cellular response to heat, mainly related to postsynaptic and protein

kinase complexes, etc., involving protein activation properties, phosphotransferase activity, alcohol groups as receptors and other molecular functions. The results of the KEGG enrichment analysis showed 192 cross-targets involving 194 pathways. The results for the top 20 signaling pathways are shown in Fig. 4, mainly involving cell signaling pathways, TNF signaling pathways, EGFR cell pathways, and other signaling pathways. Cytoscape was used to establish a complete network of compounds, targets and pathways by the 20 pathways, 40 targets and compounds revealed by the GEGG enrichment as shown in Fig. 5. Flavonoid monomeric compounds from *Callistephus chinensis* have potential therapeutic effects on diabetes.

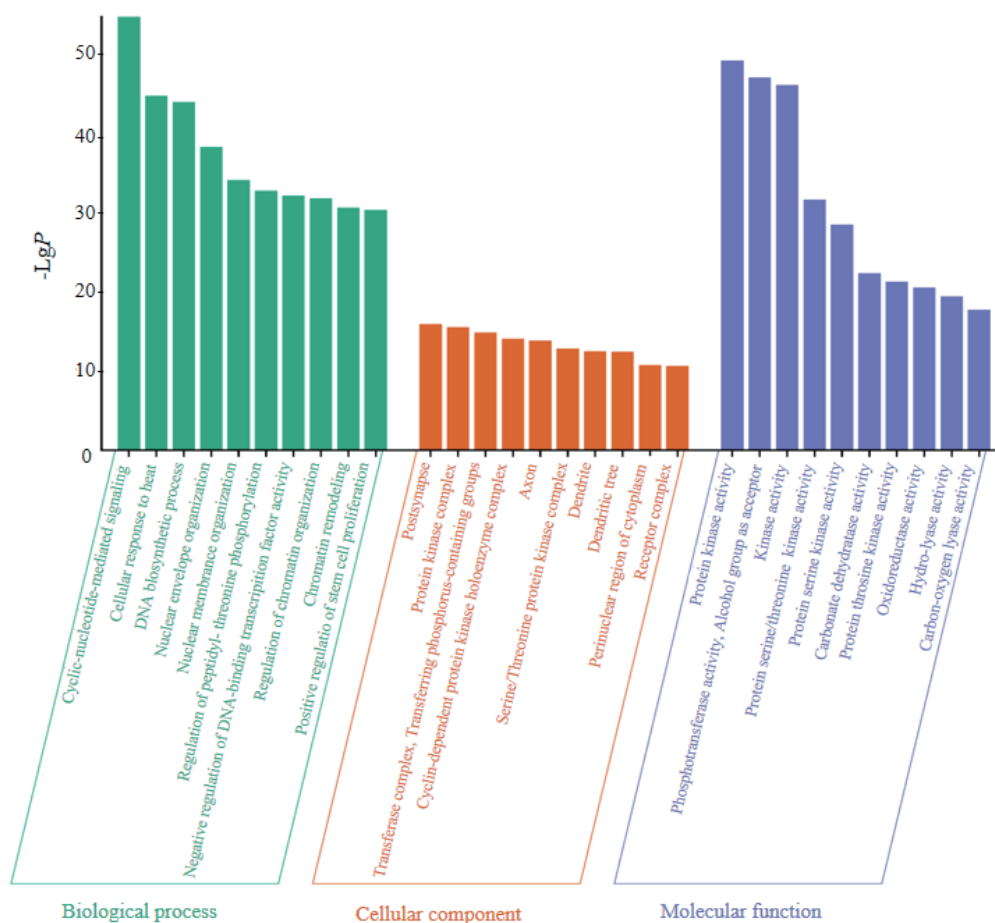


Fig. 3 GO function enrichment analysis of putative target genes GO enrichment analysis identified genes involved in GO-CC analysis, GO-BP analysis, GO-MF analysis

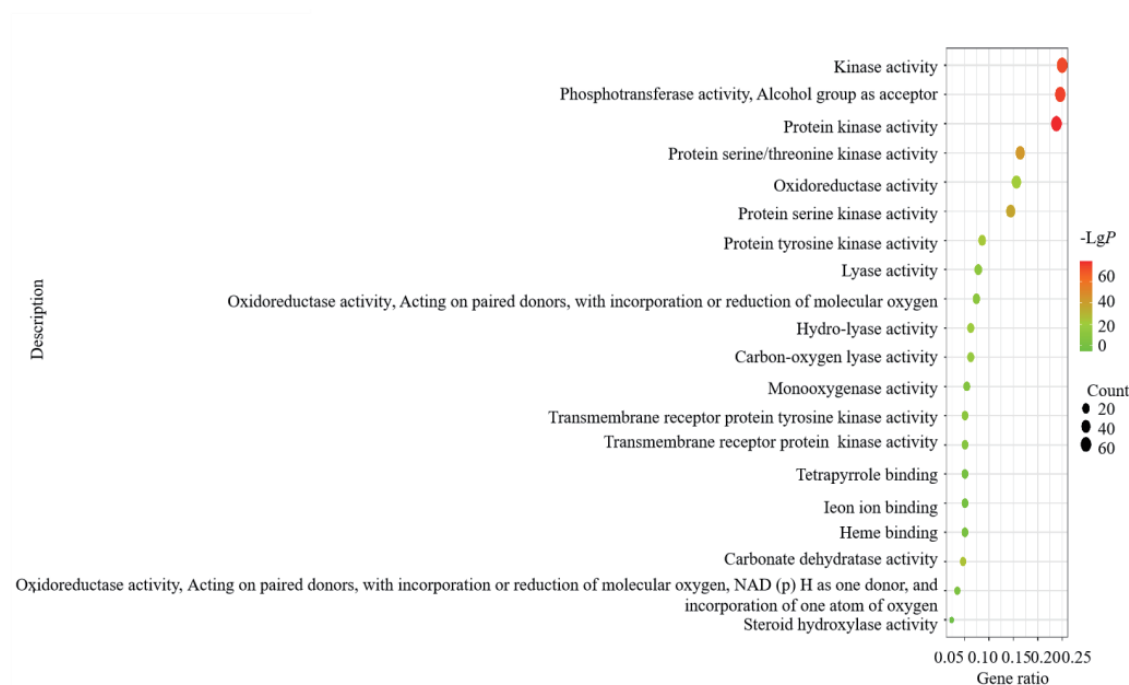


Fig. 4 KEGG pathway analysis of putative target genes

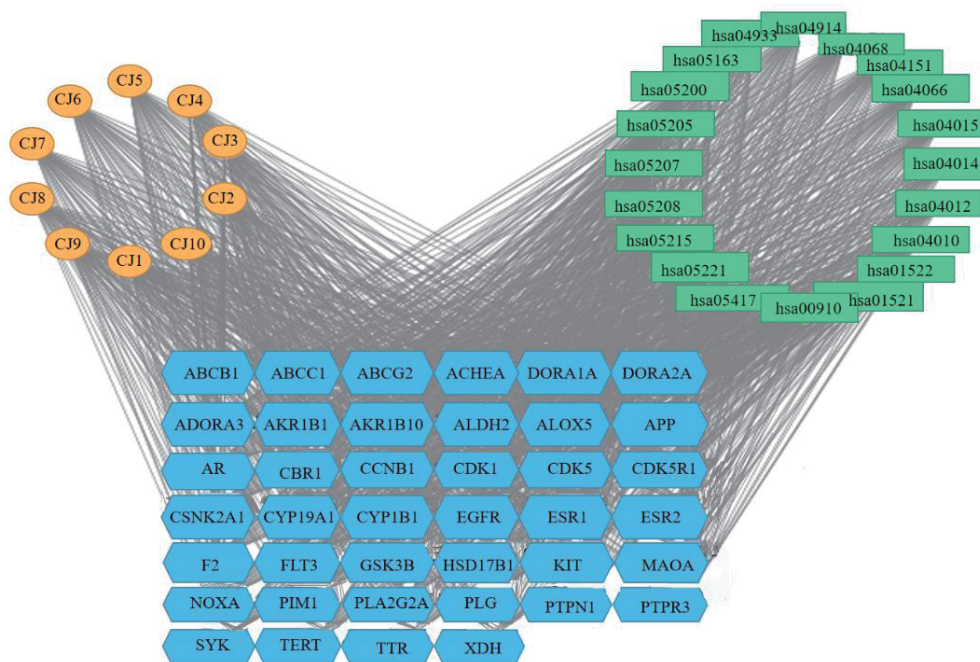


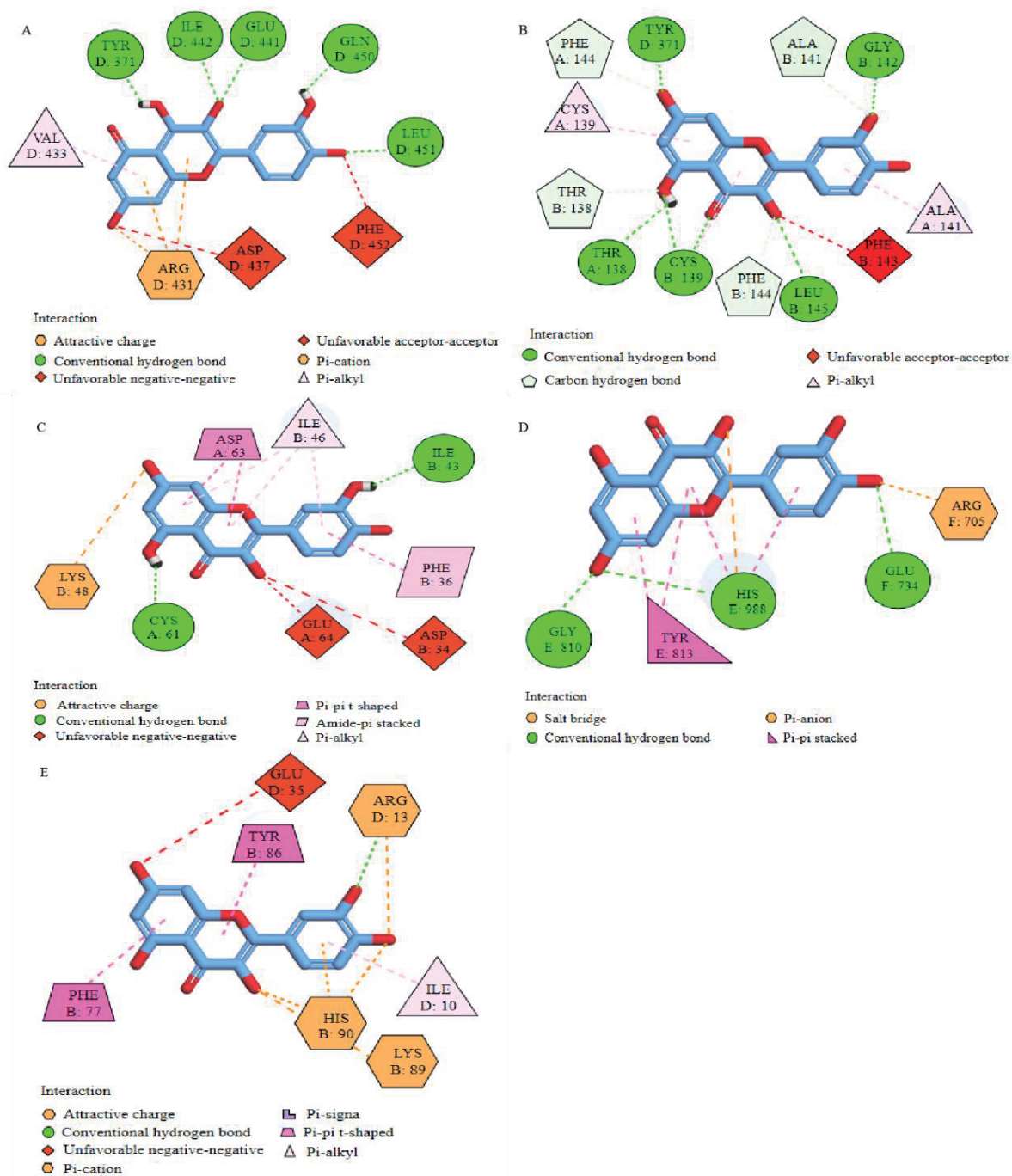
Fig. 5 “Disease-pathway-target-drug” network diagram blue nodes represent drug-disease common target, green nodes represent the pathway, and orange nodes represent the drug

In order to further clarify the binding effect between the key targets and active ingredients of *Callistephus chinensis* in the treatment of diabetes, the top 5 components of the “disease pathway-targeted drug” regulatory network medium were selected in

the PPI network analysis, and molecular docking was performed with the core targets of the top 5 degrees. It is generally believed that the higher the LibDock score, the richer the form of force, the stronger the binding ability. In this study, five target proteins were

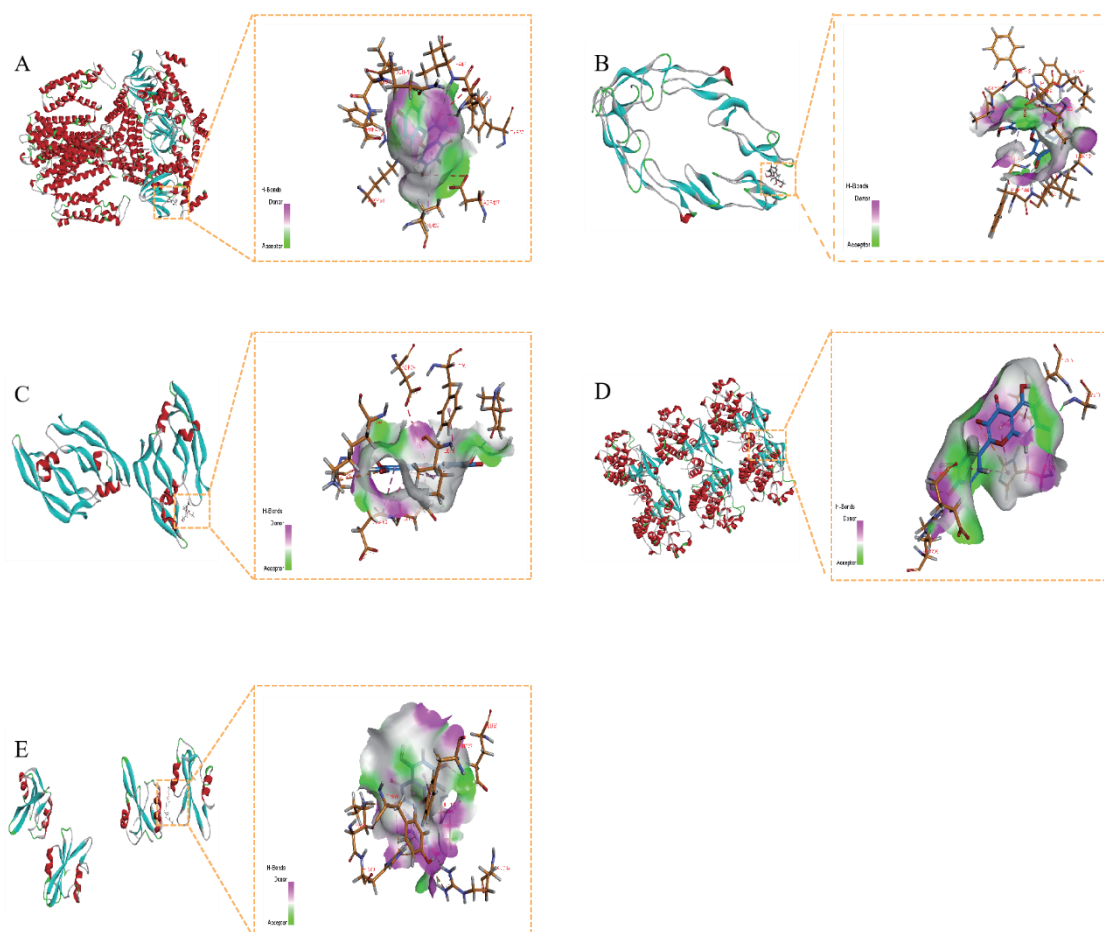
docked with ten active component molecules, and the results showed that Quercetin had relatively good interaction with TNF (114.698) and Quercetin with

AKT1 (117.104). The binding mode of the target proteins to component docking is shown in Fig. 6 and Fig. 7.



A – AKT1; B – TNF; C – VEGFA; D – EGFR; E – SRC.

Fig. 6 2D binding modes showing the interactions between Quercetin and target proteins



A – AKT1; B – TNF; C – VEGFA; D – EGFR; E – SRC.

Fig. 7 3D H-binding modes showing the interactions between Quercetin and target proteins

## 4 Discussion

The results of the analysis using Cytoscape showed that the active compounds with the highest number of flavonoid compound targets in *Callistephus chinensis* were quercetin. Studies have shown that quercetin has a wide range of pharmacological effects [11] that can improve the metabolic disorders of diabetes and effectively improve the dyslipidemia of diabetes [12]. The experimental results show that quercetin can improve insulin resistance of diabetes mellitus by reducing the expression of TNF- $\alpha$ , blocking the dysfunction of AKT1 and its downstream target protein [13], and play a role in the treatment of diabetes [12, 14]. The above results indicate that the screening of this study has a certain theoretical basis, and provides a research direction for the treatment

of diabetes mellitus with *Callistephus chinensis*. The results of molecular intercalation showed that the active components of flavonoids of *Callistephus chinensis* were well bound to the core target proteins, among which the active compound Quercetin was the best bound to AKT1, TNF, VEGFA, EGFR and SRC. In summary, active ingredients can achieve the effect of treating diabetes by modulating key targets. The enrichment analysis of GO function and KEGG pathway showed that there were 1 847 biological processes (BP), 126 cell components (CC) and 256 molecular functions (MF). Quercetin plays a role in the treatment of diabetes by regulating various pathways, mainly concentrated in cell signaling pathway, TNF signaling pathway, EGFR cell pathway and so on. Numerous studies have shown that TNF- $\alpha$  has been found to cause an increase in fatty acid levels

in fat cells, and levels of TNF- $\alpha$  cytokines are highest in patients with untreated diabetes and decrease significantly after Quercetin treatment <sup>[15]</sup>. AKT signaling pathway is the main pathway regulating glucose metabolism, which is closely related to glucose uptake, transportation and glycogen synthesis <sup>[16]</sup>. It is speculated that the main active components of flavonoids in *Callistephus chinensis* may play a role in the treatment of diabetes by reducing glucose absorption and improving inflammation.

## 5 Conclusion

In this study, a network pharmacology approach was used to initially explore the active components, core targets and signaling pathways of *Callistephus chinensis* for the treatment of diabetes, and further validate the active components and core targets by molecular docking. This study provides the basis for further experiments on the treatment of diabetes with the active components such as the flavonoids of *Callistephus chinensis*. In addition, ideas and methods for the research on the pharmacological substances of other components are provided.

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