Research Article **Open Access**

Anti-type 2 diabetes mellitus activity of *Citrus reticulata*-derived materials: Mechanistic insights from integrated network pharmacology, molecular docking, and *in vitro* assays

Hanhua Wang[1]*, Mingyue Li[1], Yanyue Wang[2]

[1]College of Traditional Chinese Medicine, Zhejiang Pharmaceutical University, Ningbo, China; [2]Department of Mathematics and Information Technology, The Education University of Hong Kong, Hong Kong, Hong Kong

***Corresponding author:** Hanhua Wang, College of Traditional Chinese Medicine, Zhejiang Pharmaceutical University, No. 666, Siming Road, Fenghua District, Ningbo, Zhejiang Province, 315100, China

Submission Date: October 16th, 2025; **Acceptance Date:** November 11th, 2025, **Publication Date:** December 9th, 2025.

Please cite this article as: Wang H., Li M., Wang Y. Anti-type 2 diabetes mellitus activity of Citrus reticulata-derived materials: Mechanistic insights from integrated network pharmacology, molecular docking, and in vitro assays. *Functional Foods in Health and Disease*. 2025; 15(12): 921 – 937. DOI: https://doi.org/10.31989/ffhd.v15i12.1802

ABSTRACT

Background: Citrus reticulata-derived materials (CRDMM) are representative medicinal and edible resources, yet their systematic evaluation for anti-type 2 diabetes mellitus (T2DM) potential remains to be fully elucidated. Objective: This study aimed to investigate the anti-T2DM potential of CRDMM and explore the underlying mechanisms of action using an integrated experimental and computational approach.

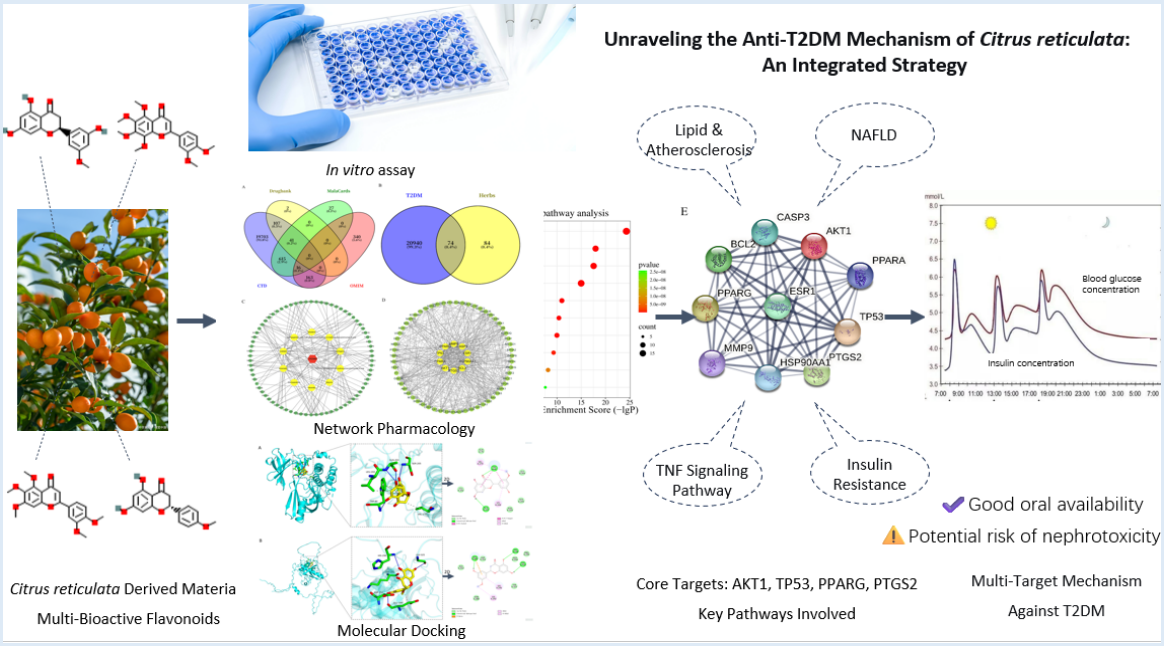
Results: *In vitro* assays demonstrated significant α-glucosidase inhibitory activity across 16 CRDMM extracts, with Guangchenpi exhibiting the strongest potency (IC_{50}: 2.5–3.8 mg/mL). Network pharmacology analysis identified 10 active compounds and 74 potential targets, among which AKT1, TP53, PPARG, and PTGS2 were highlighted as core targets via protein-protein interaction analysis. Pathway enrichment suggested involvement in lipid metabolism and inflammatory pathways. Molecular docking confirmed strong binding affinities (< –7.0 kcal/mol) between key flavonoids (e.g., neohesperidin_qt and nobiletin) and the core targets. ADMET predictions indicated favorable drug-likeness, despite potential nephrotoxicity risks.

Novelty: This study is the first to integrate multi-approach validation—combining *in vitro* bioactivity, network

pharmacology, molecular docking, and ADMET profiling—to systematically reveal that CRDMM exerts anti-T2DM effects via a multi-component, multi-target mechanism.

Conclusion: The results provide a scientific basis for developing CRDMM as functional food ingredients or dietary supplements for blood glucose management in T2DM.

Keywords: *Citrus reticulata*, type 2 diabetes mellitus, α-glucosidase inhibition, network pharmacology, molecular docking, functional food, medicinal-food homology, nobiletin



INTRODUCTION

Plants of the genus *Citrus* L. (Rutaceae) are not only widely cultivated for their fruits but also hold a significant place in traditional medicine systems, particularly in China, under the concept of "medicinal and edible homology" [1, 2]. Various of *Citrus* plants are used as traditional Chinese medicines (TCM), valued for functions such as regulating Qi, resolving phlegm, strengthening the spleen, and promoting digestion [2]. Modern scientific investigations have revealed that these materials are rich sources of bioactive compounds, including flavonoids, alkaloids, volatile oils, coumarins, and limonoids [3-10], which contribute to various health-promoting activities. For instance, extracts from Citrus fruit peels have shown auxiliary antihypertensive effects [9], while specific compounds like neohesperidin [11], naringin, naringenin [12], hesperidin [13], and tangeretin [14] exhibit potential in lowering blood glucose levels. However, the systematic mechanisms underlying the anti-diabetic effects of the complex mixtures present in specific *Citrus*-derived medicinal materials remain to be fully elucidated.

Among the diverse *Citrus* species, those derived from *Citrus reticulata* and its cultivated varieties (mandarin-type) are of particular importance in TCM. Citri Reticulatae Pericarpium (Chenpi), Citri Reticulatae Pericarpium Viride (Qingpi), and Citri Exocarpium Rubrum (Juhong) are officially recorded in the Pharmacopoeia of the People's Republic of China. As summarized in Table 1, these *C.* reticulata-derived medicinal materials (CRDMM) are obtained from different plant parts and processing stages, yet they share similar chemical compositions [15] and are widely used in clinical formulations and health products, underscoring their integrated utility.

Table 1. The Source information of CRDMM.

Name	Abbreviation	Latin name	In CP 2025?	Source plant	Part Used
Chenpi	CP	Citri Reticulatae Pericarpium	yes	*Citrus reticulata* Blanco and cultivated varieties	dried ripe peel
Geqingpi	GQP	Citri Reticulatae Pericarpium viride	yes	*Citrus reticulata* Blanco and cultivated varieties	self-falling young fruits
Guangchenpi	GCP	Citri Reticulatae Pericarpium	yes	*Citrus reticulata* 'Chachi'	dried ripe peel
Juhe	JHe	Citri Reticulatae Semen	yes	*Citrus reticulata* Blanco and cultivated varieties	dried mature seed
Juhong	JH	Citri Exocarpium Rubrum	yes	*Citrus reticulata* Blanco and cultivated varieties	dried outer pericarp
Juluo	JL	Citri Reticulatae Pericarpium Vascular	no	*Citrus reticulata* Blanco and cultivated varieties	dried vascular bundles from the pericarp
Juye	JY	Citri Reticulatae Folium	no	*Citrus reticulata* Blanco and cultivated varieties	dried leaf
Sihuaqingpi	SHQP	Citri Reticulatae Pericarpium Viride	yes	*Citrus reticulata* Blanco and cultivated varieties	peels of unripe fruits

The global prevalence of diabetes mellitus (DM), a multifactorial metabolic disorder characterized by chronic hyperglycemia, continues to rise, posing a major public health challenge [16]. Type 2 diabetes mellitus (T2DM) accounts for over 90% of all cases. Current management strategies, besides insulin, rely on oral hypoglycemic agents like biguanides and α-glucosidase inhibitors [17]. However, these drugs can be associated with adverse effects, driving the search for efficient and low-toxicity natural alternatives for auxiliary blood glucose management. Inhibiting α-glucosidase activity delays carbohydrate digestion and absorption, effectively suppressing postprandial hyperglycemia [18]-a mechanism highly relevant to carbohydrate-rich diets. This makes *in vitro* α-glucosidase inhibition a valuable preliminary screening tool for evaluating the hypoglycemic potential of natural products.

This study focuses on common CRDMM as typical " medicinal and edible homology" substances, aiming to systematically explore their potential as dietary interventions for T2DM management. We integrated in vitro activity assessment, network pharmacology, and molecular docking to evaluate their α-glucosidase inhibitory activity, identify key bioactive compounds and potential targets, and elucidate the multi-component, multi-target mode of action. Our findings provide a scientific basis for developing CRDMM into functional foods or dietary supplements beneficial for blood glucose control.

MATERIALS AND METHODS

Drugs and reagents: Information regarding the 16

batches of medicinal materials is presented in Table 2. The following drugs and reagents were used: α-Glucosidase from yeast (Batch No.: 20231112, Shanghai Shifeng Biological Technology Co., Ltd.); PNPG (4-Nitrophenyl-α-D-glucopyranoside, Shanghai Shifeng Biological Technology Co., Ltd.); Acarbose (AR grade, Shanghai Shifeng Biological Technology Co., Ltd.); 95% ethanol (AR grade, Sinopharm Chemical Reagent Co., Ltd.); Anhydrous Na_2CO_3 (AR grade, Sinopharm Chemical Reagent Co., Ltd.); and Purified water (prepared in-house).

Table 2. Sample information.

No.	Name	Manufacturer (Abbreviation)	Origin
S1	CP	Ningbo Medicinal Materials Co., Ltd. (NBMM)	Wenzhou, Zhejiang
S2	CP	Ningbo MingBEI Chinese Pharmaceutical Co. Ltd. (NBMB)	Fuzhou, Fujian
S3	GCP	NBMM	Jiangmen, Guangdong
S4	GCP	NBMB	Jiangmen, Guangdong
S5	GQP	NBMM	Wenzhou, Zhejiang
S6	GQP	NBMB	Jian, Jiangxi
S7	JH	NBMM	Qingyuan, Guangdong
S8	JH	NBMB	Maoming, Guangdong
S9	JHe	NBMM	Wenzhou, Zhejiang
S10	JHe	NBMB	Taizhou, Zhejiang
S11	JL	NBMM	Wenzhou, Zhejiang
S12	JL	NBMB	Taizhou, Zhejiang
S13	JY	NBMM	Wenzhou, Zhejiang
S14	JY	NBMB	Taizhou, Zhejiang
S15	SHQP	NBMM	Wenzhou, Zhejiang
S16	SHQP	NBMB	Jian, Jiangxi

Instruments: A Multiskan SkyHigh full-wavelength microplate reader (Thermo Fisher Scientific (China) Co., Ltd.); an SB-4200DT ultrasonic cleaner (Ningbo Scientz Biotechnology Co., Ltd.); an RE-5298 rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); an LX-2000 cooling water circulator (Beijing Changliu Scientific Instrument Co., Ltd.); a FUJ-V150 diaphragm vacuum pump (Taizhou Fujiwara Tools Co., Ltd.); an SQP electronic analytical balance (Thermo Scientific Inc.); and an HH-1 constant-temperature water bath (Guohua (Changzhou) Instrument Manufacturing Co., Ltd.).

Databases and software

Databases: The following databases were utilized in this study: the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP; http://tcmspw.com/tcmsp) [19]; the Universal Protein Resource (UniProt; https://www.uniprot.org/) [20]; the Comparative Toxicogenomics Database (CTD; http://ctdbase.org/), the DrugBank database (https://www.drugbank.ca/), the MalaCards database (https://www.malacards.org/), the Online Mendelian Inheritance in Man (OMIM) database (https://omim.org/), and the Pharmacogenomics Knowledge Base (PharmGkb) database (https://www.pharmgkb.org/) [21,22]; Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/, accessed on 30 June 2025); the STRING network platform (https://cn.string-db.org/, accessed on 30 June 2025) [23]; the GO databases (http://www.geneontology.org) and KEGG (https://www.genome.jp/kegg/); Metascape

(https://metascape.org/, accessed on 30 June 2025) [24]; the bioinformatics data analysis and visualization portal (http://www.bioinformatics.com.cn/, accessed on 30 June 2025); the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [25]; the Protein Data Bank (PDB) database (https://www.rcsb.org/) [26]; the CB-DOCK2 web server (https://cadd.labshare.cn/cb-dock2/index.php, accessed on 30 June 2025) [27]; the SwissADME online platform (http://www.swissadme.ch/, accessed on 30 June 2025); and Pro-Tox 3.0 (https://tox.charite.de/).

Software: The following software tools were employed: Cytoscape v3.10.1 (Cytoscape Consortium) [28], PyMOL 2.6 (Schrödinger, LLC), AutoDockTools-1.5.7 (Olson Laboratory), and AutoDock Vina (Olson Laboratory).

METHODS

Preparation of extracts from CRDMM and *in vitro* α-glucosidase inhibitory activity assay: Sixteen batches of Citrus herbal medicines were dried at 40 °C for 60 minutes, pulverized, and sieved through a 24-mesh sieve. Exactly 5.0 g of each sample was weighed and extracted with 50 mL of 70% ethanol under ultrasonic treatment (200 Hz) for 30 minutes. After filtration, the extraction process was repeated twice, and the resulting filtrates were combined. The combined solution was concentrated under reduced pressure and dried to obtain the crude extract. Subsequently, 60 mg of each extract was accurately weighed and dissolved in phosphate-buffered saline (PBS) to prepare sample solutions at concentrations of 4, 6, 8, 10, and 12 mg/mL. Referring to the method described in reference [14], the assay was performed in a 96-well plate. The volumes of each reagent added are listed in Table 3. Briefly, PBS buffer, α-glucosidase solution, and sample solution were sequentially added to the wells, followed by incubation at 37°C for 15 minutes. Then, 4.8 mmol/L p-nitrophenyl-α-D-glucopyranoside (PNPG) solution was added, and the mixture was incubated for another 30 minutes at a constant temperature. The reaction was terminated by adding 1 mol/L sodium carbonate (Na_2CO_3) solution. The absorbance was measured at a wavelength of 405 nm. The inhibitory rate against α-glucosidase was calculated using equation (1):

$$\text{Inhibition rate (\%)} = [1 - (A3 - A4) / (A1 - A2)] \times 100$$

Where A1, A2, A3, and A4 represent the absorbance of the sample group, sample blank group, negative control group, and blank group, respectively. The positive control group (A5) was prepared by replacing the sample solution with acarbose solution, while other steps remained consistent with Table 3.

Table 3. Reaction system for the α-glucosidase inhibitory activity assay.

Group	PBS（μL）	Enzyme（μL）	Sample（μL）	PNPG（μL）	Na2CO3（μL）
A1	120	15	15	30	60
A2	135	–	15	30	60
A3	135	15	–	30	60
A4	150	–	–	30	60
A5	120	15	15	30	60

Notes: - indicates that the reagent was not added; the concentration of the acarbose solution was 0.5 mg/mL.

Network pharmacology

Screening of active compounds and protein targets: Active compounds of CRDMM were retrieved from the TCMSP database. Compounds with higher activity were further screened based on the criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18.

Target proteins corresponding to these active compounds were obtained from the TCMSP database and converted into standardized gene names using the UniProt database.

Collection of T2DM-related targets: T2DM-related targets were acquired from four databases: CTD, DrugBank, MalaCards, and OMIM. After removing duplicates and false-positive targets, the overlapping targets between Citrus herbal medicine targets and T2DM-related targets were identified using the Venny 2.1 online tool. A network comprising 10 active compounds and 74 overlapping targets was visualized using Cytoscape v3.10.1. Topological analysis was performed to calculate the degree value of each node in the network.

PPI network and core target analysis: To explore the potential key targets of CRDMM and their interactions, a protein-protein interaction (PPI) network was constructed using the STRING database platform. The species was set to "Homo sapiens," and the confidence threshold was set to medium (0.400). Finally, the CytoHubba plugin was used to rank the targets by maximal clique centrality (MCC) method, and the top 10 hub targets were identified as core targets from the PPI network.

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis: Biological functions of the core targets were systematically annotated using the Gene Ontology (GO) database, including cellular components (CC), molecular functions (MF), and biological processes (BP), to provide an overview of the enriched biological functions, pathways, and cellular locations. The KEGG database, a core functional genomics resource integrating genomic, chemical, and systems biological data, was employed for pathway enrichment analysis. Based on the preprocessed core targets, GO and KEGG analyses were performed using Metascape to interpret functional characteristics related to CC, MF, BP, and signaling pathways. The results were imported into the bioinformatics data analysis and visualization website to generate bubble plots.

Molecular docking and ADMET analysis: The top four bioactive components from CRDMM based on degree value were selected, and their three-dimensional molecular structures (in *Mol2 format) were obtained from the PubChem database. The three-dimensional structures (in *PDB format) of the four target proteins with the highest scores were downloaded from the PDB database. Molecular docking was performed using the CB-Dock2 online server to calculate binding energies and predict drug–target interactions. The docking results were visualized and analyzed using PyMOL and AutoDock software.

SwissADME was employed to predict drug-like properties. The bioactive components of citrus TCM were input into the server for analysis of ADMET characteristics. Pro-Tox 3.0 (https://tox.charite.de/) was used to predict the toxicity of these components.

Data processing: Data from the *in vitro* enzyme activity assays are expressed as mean ± standard deviation ($\bar{X}$ ± S) and were processed using OriginPro 2021.

RESULTS

Determination of α-glucosidase inhibitory activity of CRDMM *in vitro*: α-Glucosidase can hydrolyze glycosidic bonds to release glucose. Inhibiting its activity affects glucose conversion, thereby significantly reducing blood glucose levels and achieving hypoglycemic effects [29]. The inhibition rates and IC_{50} values of 16 CRDMM

samples were calculated using equation (1), and the results are presented in Table 4. As shown in Table 4, all 16 batches of CRDMM exhibited inhibitory effects on α-glucosidase *in vitro*. The results demonstrate that S3 and S4 (GCP) showed the highest inhibitory activity against α-glucosidase across all tested concentrations.

Table 4. α-Glucosidase inhibition rates and IC_{50} values of 16 CRDMM samples.

No.	Inhibition Rate (%, mean ± sd)					IC_{50} (mg/mL)
	4.0 mg/mL	6.0 mg/mL	8.0 mg/mL	10.0 mg/mL	12.0 mg/mL	
S1	33.21±1.12	44.17±0.92	55.63±0.09	64.27±0.36	72.88±0.78	7.2
S2	24.69±1.48	34.83±0.92	43.27±0.80	49.11±1.33	58.43±1.32	9.9
S3	50.17±1.22	58.34±0.99	66.39±1.11	72.75±0.85	79.36±1.21	3.8
S4	54.90±1.08	62.68±1.14	70.82±1.69	77.34±1.56	83.25±0.13	2.5
S5	28.35±1.28	35.11±0.98	44.26±1.39	54.85±1.13	64.90±0.23	9.0
S6	42.64±0.43	52.05±0.35	60.33±0.87	68.29±1.68	75.08±0.92	5.6
S7	35.84±1.30	46.03±0.65	57.25±1.28	66.35±1.07	73.39±0.66	6.8
S8	42.53±1.83	52.24±0.32	60.59±0.37	68.81±1.73	75.90±0.88	5.6
S9	10.34±0.33	18.74±1.51	25.83±0.64	33.59±0.81	41.69±0.21	14.2
S10	20.35±0.86	30.57±0.83	39.19±0.29	47.47±1.36	54.52±0.78	10.7
S11	7.20±0.99	15.82±1.36	23.48±1.10	30.09±1.36	37.58±1.96	15.2
S12	11.08±1.23	19.54±0.14	27.99±0.45	35.77±1.56	43.28±1.34	13.6
S13	27.54±1.15	38.07±0.65	47.24±1.10	56.74±1.82	67.50±1.04	8.5
S14	29.42±1.62	39.59±0.59	50.84±0.23	58.19±1.68	65.15±1.78	8.3
S15	26.37±1.23	34.93±1.96	42.64±0.66	53.23±1.60	62.06±1.64	9.4
S16	27.18±0.12	34.74±0.66	43.48±0.89	53.48±0.48	63.85±0.78	9.2

Network pharmacology prediction results

Screening of active compounds and related targets in CRDMM: Initially, a total of 10 active ingredients from CRDMM and 158 related targets were identified from the TCMSP database based on OB and DL criteria. After removing duplicates, 21,014 disease targets were obtained, as illustrated in Figure 1-A. There were 74 overlapping targets between the active ingredients of CRDMM and T2DM, as shown in Figure 1-B. The relationships between 10 active ingredients of CRDMM and the 74 overlapping targets involved in the intervention of T2DM were visualized using Cytoscape software, presented in Figure 1-C.

Construction of PPI network and identification of core targets: To explore the molecular mechanisms of CRDMM against T2DM, the 74 overlapping targets were imported into the STRING database to construct a PPI network. The resulting network consisted of 71 nodes and 535 edges, with an average node degree of 15.1. The PPI network built on the STRING platform was then imported into Cytoscape for visualization, as shown in Figure 1-D. Finally, the top 10 core targets were selected in descending order of degree using the Cytohubba plugin. These included AKT serine/threonine kinase 1 (AKT1), tumor protein p53 (TP53), peroxisome proliferator activated receptor gamma (PPARG), prostaglandin-endoperoxide synthase 2 (PTGS2), B-cell lymphoma 2 (BCL2), among others, as depicted in Figure 1-E.

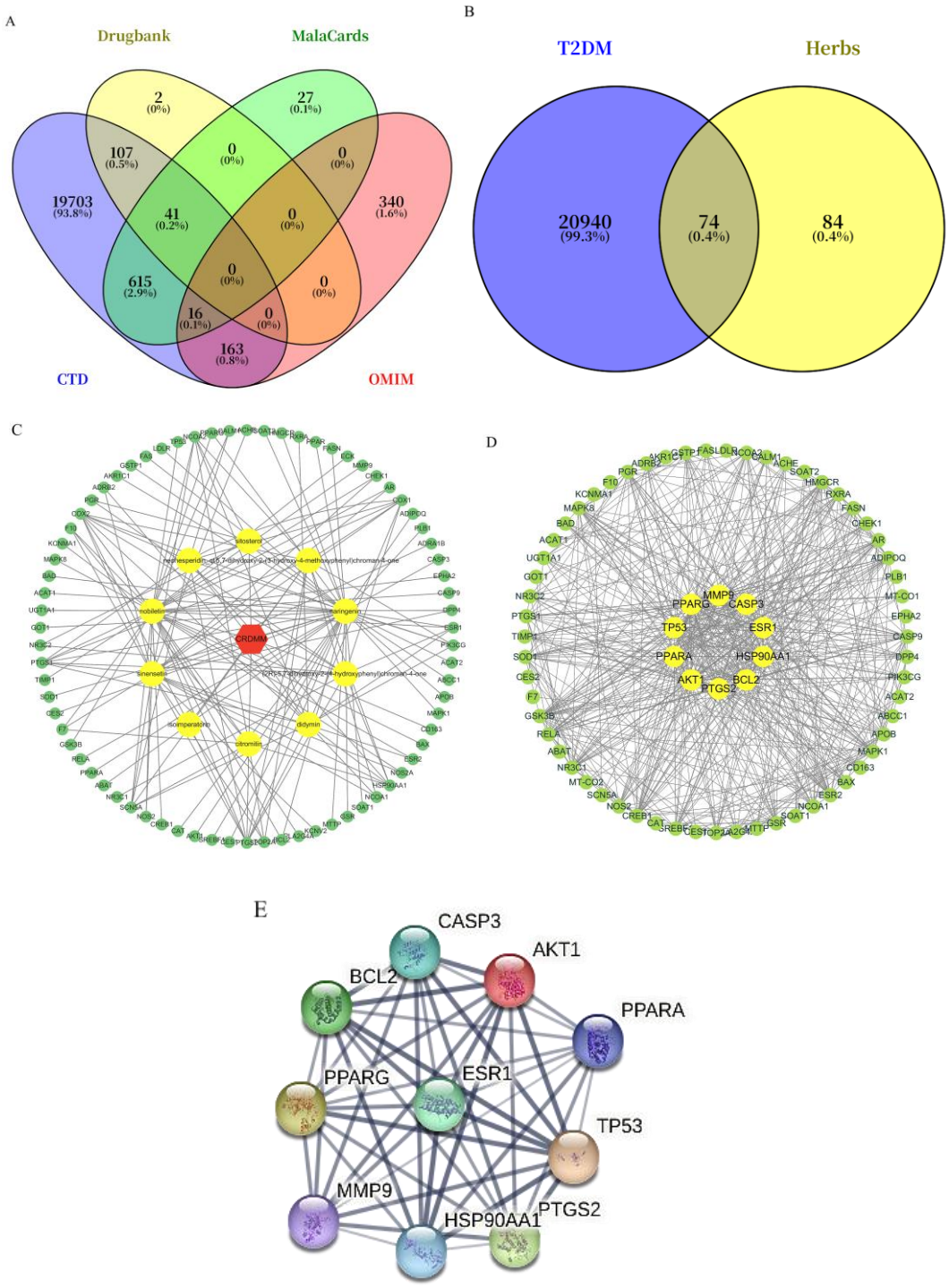


Figure 1. Screening of core targets of CRDMM against T2DM.

A Venn diagram of T2DM targets collected from different databases.

B Venn diagram of the overlapping targets between CRDMM and T2DM.

C PPI network of CRDMM active ingredients and disease targets (Yellow and green nodes represent the active compounds and disease targets, respectively).

D PPI network of the common target genes (The inner-to-outer arrangement of the circle indicates the strength of protein binding interactions, and the connecting lines represent PPIs).

E The core targets identified by CytoHubba.

GO and KEGG pathway enrichment analysis: Using the Metascape database, GO and KEGG enrichment analyses were performed on the 74 overlapping targets, and the results were visualized via a bioinformatics mapping website. As shown in Figure 2-A to 2-C, only the top 10 entries in BP, CC, and MF are displayed in the GO enrichment analysis. KEGG pathway analysis displayed the top 10 enriched pathways, including Lipid and atherosclerosis, Non-alcoholic fatty liver disease, Chemical carcinogenesis - receptor activation, Pathways of neurodegeneration - multiple diseases, and TNF signaling pathway, among others, as illustrated in Figure 2-D.

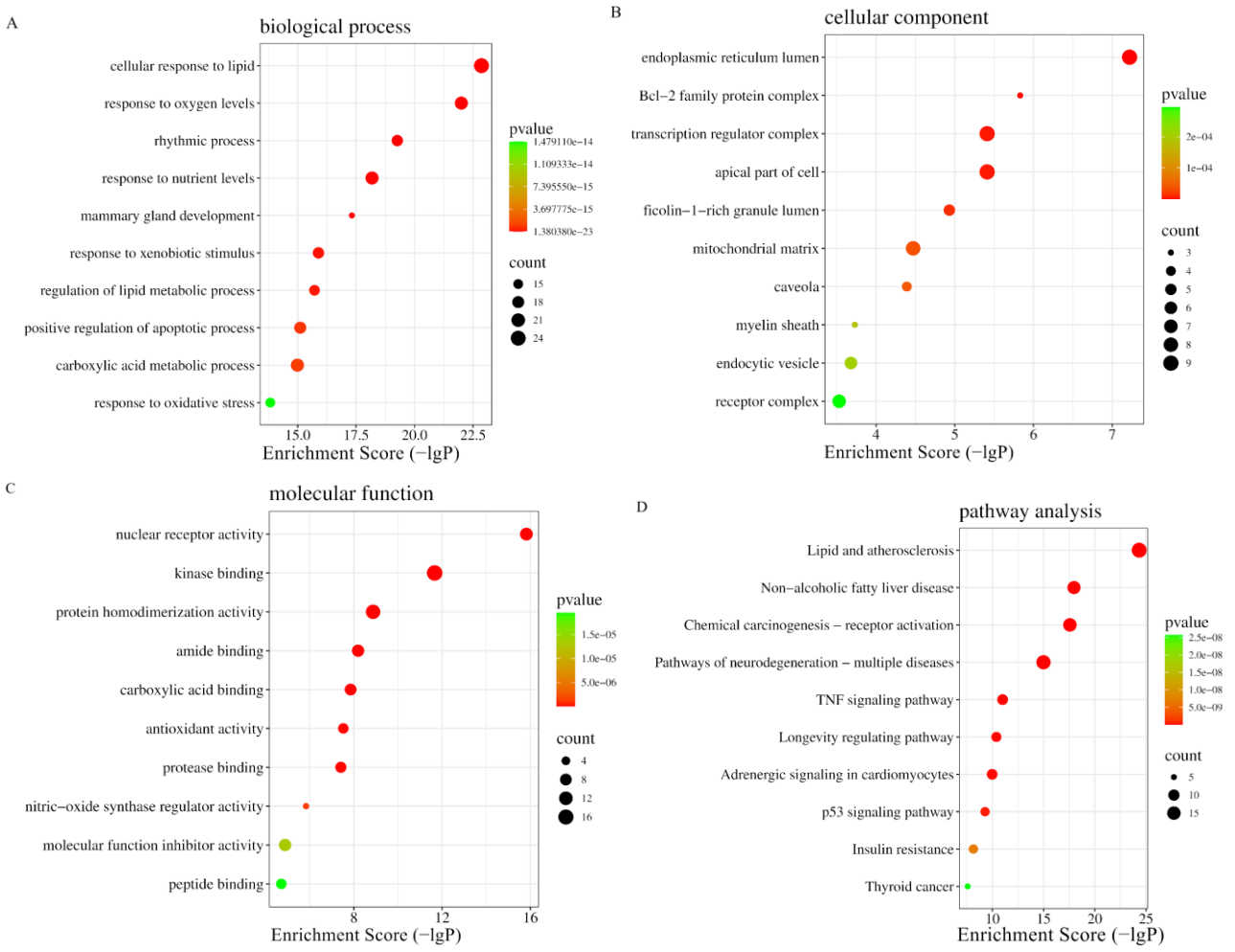


Figure 2. Enrichment analyses of GO and KEGG

A Top 10 biological processes by enrichment degree in GO analysis.

B Top 10 cellular components by enrichment degree in GO analysis.

C Top 10 molecular functions by enrichment degree in GO analysis.

D Top 10 signaling pathways by enrichment degree in KEGG analysis.

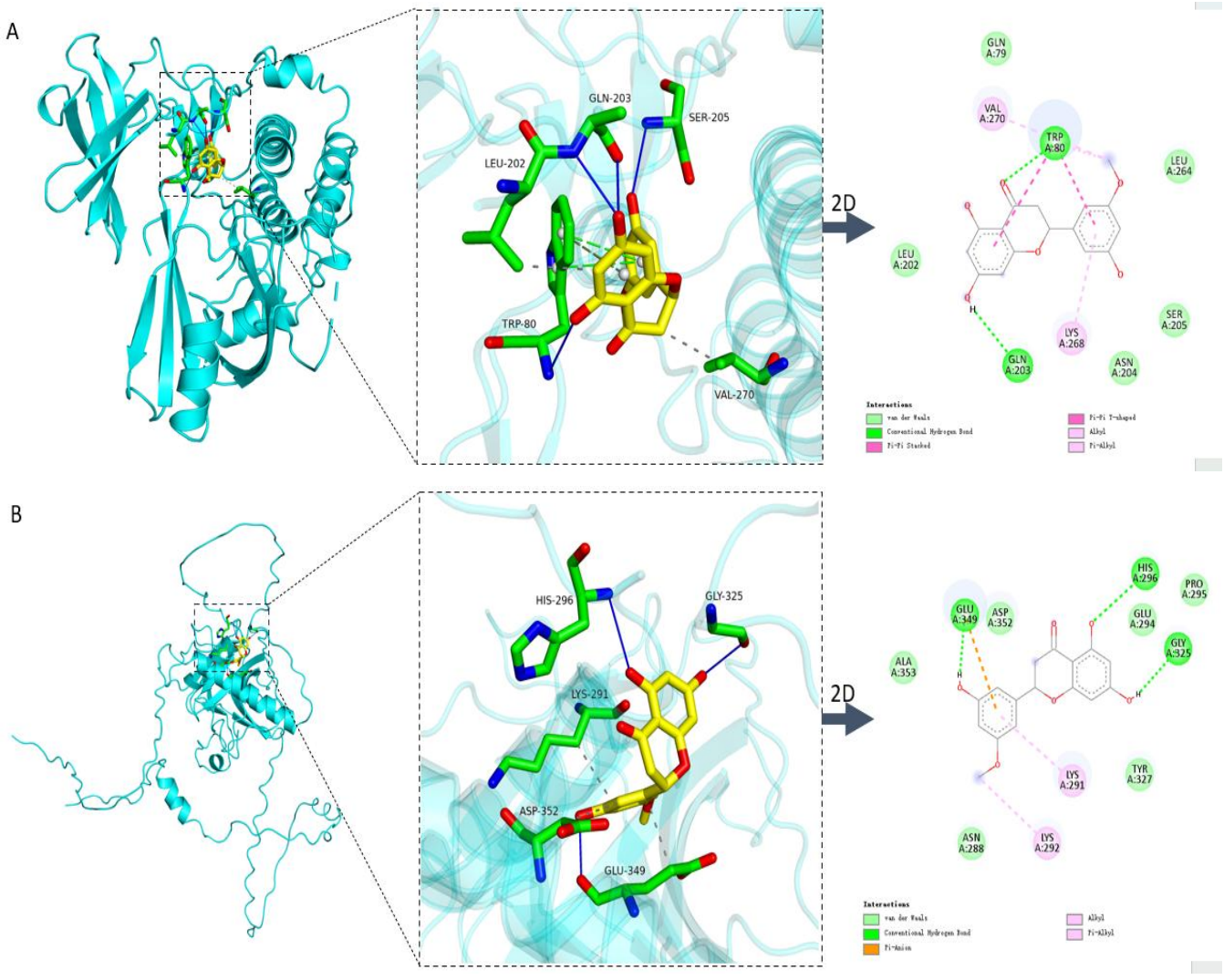
Molecular docking verification and ADMET analysis: Based on the aforementioned core targets and Cytoscape visualization results, the binding energies between the active components of citrus traditional Chinese medicine—neohesperidin_qt, nobiletin, sinensetin, and didymin—and the T2DM-related core targets (AKT1,

TP53, PPARG, PTGS2) were predicted and calculated. A binding free energy of < -7.0 kcal/mol is generally considered to indicate strong binding activity between the ligand and receptor [30], as shown in Table 5. The results demonstrate that the binding energies between the key active components of citrus traditional Chinese medicine and the core targets are all < -7 kcal/mol, indicating that these components play a critical role in the disease. The molecular docking results of neohesperidin_qt with the target exhibiting the highest binding energy were visualized using PyMol software, as illustrated in Figure 3.

Table 5. Binding energies between active components of CRDMM and core targets.

Target	Binding energy (kcal/mol)			
	neohesperidin_qt (MOL001798)	Nobiletin (MOL005828)	sinensetin (MOL001803)	Didymin (MOL005849)
AKT1	-9.6	-8.9	-9.2	-9.2
TP53	-7.9	-7.9	-7.6	-7.4
PPARG	-8.6	-7.8	-8.3	-8.2
PTGS2	-9.7	-8.9	-9.1	-9.1

Notes: The compound identifiers in parentheses (e.g., MOL001798) correspond to the TCMSP database accession numbers. The same identifiers are used consistently throughout all tables for each compound.



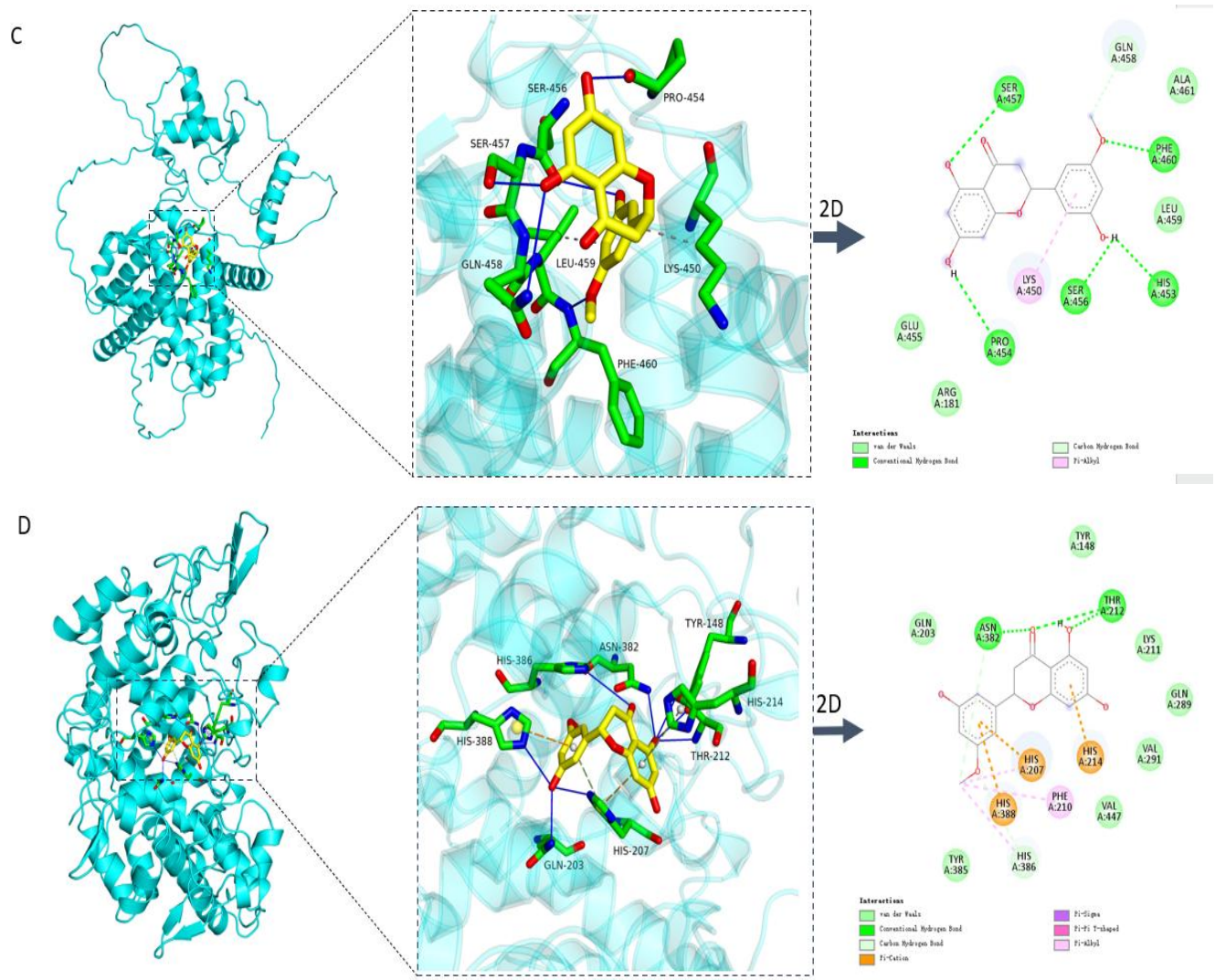


Figure 3. Molecular docking diagrams of neohesperidin_qt with core targets of T2DM.

A Neohesperidin_qt dock with AKT1.

B Neohesperidin_qt dock with TP53.

C Neohesperidin_qt dock with PPARG.

D Neohesperidin_qt dock with PTGS2.

ADMET property evaluation is an indispensable core assessment module in the research and development process of natural medicines [31]. ADMET analysis was performed using the SwissADME database, and the results indicated that the key active components of citrus traditional Chinese medicine exhibit favorable pharmacokinetic properties. As shown in Table 6, the four active components demonstrated no side effects in various predictive models, including gastrointestinal absorption, blood-brain barrier penetration, P-glycoprotein substrate susceptibility, and human oral absorption. Furthermore, all four active components showed good biocompatibility in various toxicity prediction models, with no indications of reverse mutation or hepatotoxicity. However, all four compounds were predicted to be "active" for nephrotoxicity.

Table 6. ADMET analysis of key active components of CRDMM.

Property	neohesperidin_qt (MOL001798)	nobiletin(MOL005828)	sinensetin (MOL001803)	Didymin (MOL005849)
GI absorption	high	high	high	high
BBB permeant	no	no	yes	yes
P-gp substrate	yes	no	no	no
CYP1A2 inhibitor	yes	no	no	yes
CYP2C19 inhibitor	no	no	yes	yes
CYP2C9 inhibitor	no	yes	yes	no
CYP2D6 inhibitor	no	no	no	no
CYP3A4 inhibitor	yes	yes	yes	yes
Predicted LD50 (mg/kg)	2000	5000	5000	2000
Hepatotoxicity	inactive	inactive	inactive	inactive
Nephrotoxicity	active (0.64)	active (0.51)	active (0.51)	active (0.64)
Carcinogenicity	inactive	inactive	inactive	inactive
Mutagenicity	inactive	inactive	inactive	inactive
Cytotoxicity	inactive	inactive	inactive	inactive

Notes: The numbers in parentheses represent the probability values (ranging from 0 to 1) of predictive confidence. A value greater than 0.5 but less than 0.7 indicates a marginal prediction by the model, suggesting potential risk of the molecule exhibiting this toxicity; a value greater than 0.7 serves as a high-risk alert.

DISCUSSIONS

T2DM is a prevalent global metabolic disease, underscoring an urgent need to explore safe and effective alternative therapeutic strategies. This study integrated *in vitro* activity evaluation, network pharmacology, molecular docking, and ADMET prediction to systematically elucidate the potential and mechanisms of CRDMM against T2DM. These materials embody the concept of "medicinal and edible homology," an inherent attribute that makes them ideal candidates for development into dietary-based preventive strategies or functional food ingredients.

The study of CRDMM as an integrated entity is justified by their shared chemical foundation [32, 33] and traditional use in TCM for regulating qi and strengthening the spleen [34]. Our *in vitro* α-glucosidase inhibition assays showed that all tested CRDMM extracts exhibited varying degrees of inhibitory activity. Notably, GCP demonstrated the strongest inhibitory effect, suggesting its superior potential in assisting blood glucose management. The efficacy differences among various CRDMMs may be attributed to their distinct chemical profiles, which are influenced by factors such as cultivar, processing methods, and geographical origin [15]. The strong activity of GCP warrants further investigation into its specific compositional content, particularly polymethoxyflavones (PMFs) with high bioactivity, such as tangeretin and nobiletin [32,35]. These findings provide a scientific basis for selecting specific CRDMMs, like GCP, as potent natural sources for functional foods targeting postprandial hyperglycemia.

PPI network and subsequent topological analysis highlighted AKT1, TP53, PPARG, and PTGS2 as core targets. AKT1 is a central node in the insulin signaling pathway and is crucial for glucose uptake [36]. PPARG is a key regulator of adipocyte differentiation and lipid metabolism, and is also the target of thiazolidinedione insulin sensitizers [37]. TP53, although widely known for its tumor suppressor function, is also involved in the regulation of cellular metabolism [38]. PTGS2 is a major

mediator of inflammation, a well-established pathological factor in insulin resistance and T2DM progression [39]. The involvement of these diverse targets suggests that CRDMM may ameliorate T2DM by synergistically improving insulin sensitivity, modulating lipid metabolism, and alleviating chronic inflammation. This multi-faceted action is highly advantageous for a dietary intervention, as it addresses the complex pathophysiology of T2DM holistically.

KEGG pathway enrichment analysis further supported this perspective. Key enriched pathways such as "Lipid and atherosclerosis", "Non-alcoholic fatty liver disease ", and "TNF signaling pathway" closely link the pharmacological effects of CRDMM to the complex metabolic-inflammatory network underlying T2DM and its common comorbidities. This indicates that the therapeutic value of CRDMM may extend beyond blood glucose control to improving overall metabolic health. This aligns with the growing consumer interest in foods that offer comprehensive metabolic benefits.

The strong binding energies (< -7.0 kcal/mol) predicted by molecular docking between key flavonoids (neohesperidin_qt, nobiletin, sinensetin, didymin) and the core targets provided molecular-level evidence for the network pharmacology predictions. Notably, neohesperidin_qt and nobiletin exhibited particularly high affinity towards AKT1 and PTGS2, suggesting their pivotal roles in mediating the insulin-sensitizing and anti-inflammatory effects of CRDMM. These compounds could serve as key quality control markers for evaluating CRDMM-based health products.

From a drug development perspective, the predicted ADMET properties of these key compounds are encouraging. Their high gastrointestinal absorption and drug-likeness suggest good oral bioavailability, which is crucial for their application as functional foods or oral therapies. However, the predicted nephrotoxicity (albeit computational) warrants caution. This highlights the importance of further *in vivo* toxicological studies prior to clinical translation to ensure safety. For food applications, long-term safety under conditions of daily consumption is paramount and requires thorough assessment.

This study presents a scientific innovation by systematically integrating in vitro bioactivity screening with comprehensive computational frameworks—network pharmacology, molecular docking, and ADMET profiling—to elucidate the anti-T2DM potential of CRDMM. Our approach not only identified Guangchenpi (GCP) as the most potent α-glucosidase inhibitor among the CRDMM but also provided novel mechanistic insights by revealing a multi-component, multi-target network. The prediction of key targets like AKT1 and PPARG moves beyond the conventional single-target view, offering a holistic understanding of how CRDMM may concurrently regulate blood glucose, lipid metabolism, and inflammation.

The findings hold significant practical implications for multiple stakeholders. For researchers and the nutraceutical industry, the marked efficacy of GCP provides a evidence-based rationale for selecting superior CRDMM varieties for product development. The identified key bioactive compounds (e.g., nobiletin, neohesperidin_qt) can serve as chemical markers for standardizing CRDMM extracts to ensure quality and efficacy. The immediate next translational step involves validating these findings in more human-relevant systems, such as employing mammalian α-glucosidase assays and human cell models, to bridge the gap between bench-side discovery and the development of effective, evidence-based functional foods for T2DM management.

While this study provides valuable insights into the anti-T2DM potential of CRDMM, we acknowledge several limitations that also chart the course for future research.

Firstly, there is an apparent disconnect between our in silico predictions, which suggested multi-target actions on key nodes like AKT1 and PPARG, and our in vitro validation, which was confined to α-glucosidase inhibition. We recognize that the potent α-glucosidase inhibitory activity, particularly of GCP, directly supports a role in managing postprandial hyperglycemia but does not experimentally validate the broader, systemic mechanisms proposed by network pharmacology. Secondly, the use of a yeast-derived α-glucosidase, while a standard preliminary screening tool, may not fully recapitulate the activity against mammalian intestinal enzymes, and the physiological relevance of the effective concentrations in vivo requires further pharmacokinetic studies. Thirdly, the molecular docking was based on the presumed presence of key flavonoids (e.g., nobiletin) in our extracts, yet a comprehensive quantitative chemical profiling of the tested CRDMM batches was beyond the scope of this work. Consequently, we have tempered our conclusions to highlight the confirmed carbohydrate-digestion inhibition pathway, while presenting the predicted effects on insulin signaling and lipid metabolism as compelling hypotheses. Future investigations will focus on: 1) quantifying the key active constituents in the most potent extracts (like GCP) using LC-MS; 2) validating the multi-target hypotheses through in vitro cell-based assays (e.g., glucose uptake in insulin-resistant cells) and in vivo studies; and 3) employing more physiologically relevant enzyme models to strengthen the translational value of our findings.

CONCLUSION

This study employed an integrated multidisciplinary approach to preliminarily investigate the potential anti-T2DM mechanisms of CRDMM. In vitro assays confirmed the significant α-glucosidase inhibitory activity of all tested CRDMM extracts, among which GCP demonstrated the most potent effect, highlighting its superior potential for managing postprandial hyperglycemia. Network pharmacology analysis constructed a "compound-target-pathway" network, predicting that core targets like AKT1, PPARG, and PTGS2 might mediate the effects of CRDMM through pathways related to insulin signaling, lipid metabolism, and inflammation. Molecular docking suggested strong binding affinities between key flavonoids (e.g., nobiletin, neohesperidin_qt) and these core targets, providing a structural basis for the predicted multi-target mechanisms.

However, it is acknowledged that these integrated mechanisms remain predictive and require further experimental validation. The favorable ADMET profiles predicted for the active compounds support their potential for oral administration.

Overall, this work provides a modern scientific interpretation of the 'medicinal and edible homology' concept and offers a theoretical foundation for developing CRDMM, particularly GCP, into functional foods or nutraceuticals for T2DM management. Future studies should prioritize the quantification of bioactive compounds in GCP, alongside in vitro cellular and in vivo validation of the predicted multi-target hypotheses to bridge the gap between computational prediction and clinical application.

Conflict of interest statement: The authors declare no conflicts of interest.

Contributions: Wang, H. conceptualization, methodology, software, writing - original draft. Li, M. data curation, supervision, visualization. Wang,Y. resources, investigation, writing - reviewing and editing.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Support: Supported by General scientific research project of Zhejiang Education Department (No. Y202147756).

REFERENCES

1. Stavrakakis I, Melidis P, Kavroulakis N, Goliomytis M, Simitzis P, Ntougias S. Bioeconomy-based approaches for the microbial valorization of Citrus processing waste. *Microorganisms*. 2025;13(8): 1891–1891. DOI: http://doi.org/10.3390/microorganisms13081891.
2. Cao X, Pan S. Progress in research on secondary metabolites and biological activity of medicinal and edible citrus plants. *Food Sci*. 2022;43(23):305–315. DOI: http://doi.org/10.7506/spkx1002-6630-20220627-302.
3. Lai C, Huang M, Xiong Q, Liang Y, Jiang Y, *Zhang* J. Green and efficient approach to extract bioactive flavonoids with antioxidant, antibacterial, antiglycation, and enzyme inhibitory activities from navel orange peel. *Sus Chem Pharm.* 2024; 38:101479. DOI: http://doi.org/ 10.1016/J.SCP.2024.101479.
4. Yang F, Tan X, Chu S, Yin M, Zhang Z, Peng H. UPLC-Q-TOF-MS with chemometrics approach analysis of nonvolatile compounds for medicinal Citrus reticulata with cultivar and areas variations. *Phytochem Anal*. 2025;36(2):467–484. DOI: http://doi.org/10.1002/pca.3496.
5. Ning X, Luo W, Li J, Chen D, Qiu Z, Lin, Z, *et al*. Compatibility relationship of Citri Reticulatae Pericarpium Viride-Aurantii Fructus drug pair in the treatment of dyskinetic gastrointestinal diseases based on network pharmacology and animal experiments. *Nat Prod Res Dev*. 2024;36(12):2133–2144. DOI: http://doi.org/10.16333/j.1001-6880.2024.12.014.
6. Sui L, Chen W, Fan S, Xu W. Content analysis of nine components in different processed products of Juhong (Citri Eoxcarpium Rubrum) by LC - MS/MS. *Chin Arch Tradit Chin Med*. 2023;41(11):226–229,293–294. (in Chinese) DOI: http://doi.org/10.13193/j.issn.1673-7717.2023.11.042.
7. Lin X, Chen X, Meng J, Zhou S, Sun Y, Wang S. Screening quality markers of antioxidant activity of Citri Reticulatae Semen based on spectrum-effect relationship. *J Chin Med Mater*. 2023;46(1):130–136. (in Chinese) DOI: http://doi.org/10.13863/j.issn1001-4454.2023.01.023.
8. Liu M, Yu X, Qin C, Zhao Q, Wang S, Wang H. Green and efficient extraction of Aurantii Fructus Immaturus flavonoids by natural deep eutectic solvents: Component identification, extraction mechanism and biological activity. *J Mol Liq.* 2025;437128543. DOI: http://doi.org/ 10.1016/J.MOLLIQ.2025.128543.
9. Yan P, Zhang Y, Zhang H, Zhang h, Zheng M, Chai X. Stoichiometry analysis on inhibition of α-glycosidase activity of Citrus peel. *Food Sci Technol*. 2024;49(6):239–246. (in Chinese) DOI: http://doi.org/10.13684/j.cnki.spkj.2024.06.032.
10. Li J, Zou S, Yang W, Peng M, Chen B, Deng J, *et al*. Identification of volatile and nonvolatile compounds in Citri Reticulatae Pericarpium Viride using GC-MS, UPLC-Q-Exactive Orbitrap-MS, and HPLC-PDA. *Food Sci Nutr*. 2023;11(3):1415–1425. DOI: http://doi.org/10.1002/fsn3.3181.
11. Hajra D, Chouni A, Ray R, Paul S. Neohesperidin, a flavanone glycoside resourced from Curcuma amada rhizome, holds the key to the remediation of diabetes mellitus: An integrated experimental and computational study. *Pharmacol Res-Mod Chin Med*. 2024; 11:100430. DOI: http://doi.org/10.1016/j.prmcm.2024.100430.
12. Fotouhi S, Yavari A, Bagheri A R, Askari V R, Gholami Y, Rahimi V B. Exploring the promising impacts of naringin and its aglycone constituent naringenin as major citrus flavonoids on diabetes and its complications. *J Funct Foods*. 2025; 124:106643. DOI: http://doi.org/10.1016/j.jff.2025.106643.
13. Mi H, Shi X, Yang Z. Effect of hesperedin on glucose metabolism in insulin-resistant HepG2 cells. *Northwest Pharm J*. 2013;28(3):278–281. (in Chinese) DOI: http://doi.org/10.3969/j.issn.1004-2407.2013.03.021.
14. Zhang Y, Yan P, Zhang H, Zhang H, Zheng M, Chai X. Based on bio-network analysis to study the adjunctive preventive effects of Citrus fruit peels on type 2 diabetes mellitus. *Sci Technol Food Ind*. 2024;45(5):319–330. (in Chinese) DOI: http://doi.org/10.13386/j.issn1002-0306.2023010158.
15. Wu M, Ma S, Zhong C, Wu M, Zhang Y, Cao H. *et al*. Flavonoids variations in five medicinal materials of genus Citrus. *Chin Tradit Patent Med*. 2022;44(10):3225–3228. DOI: http://doi.org/10.3969/j.issn.1001-1528.2022.10.043.
16. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K,

Duncan B B, *et al*. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022; 183:109119.
DOI: http://doi.org/10.1016/j.diabres.2021.109119.

17. Khimmaktong W, Matsathit U, Nuinamwong S, Sermwittayawong D, Chubuathong P, Sukpondma Y. Polysaccharides from split gill mushrooms (Schizophyllum commune Fr.) improve glucose metabolism and reduce muscleinflammation in a rat model of type 2 diabetes. *Funct Foods Health Dis*. 2025;15(8):519–539.
DOI: https://doi.org/10.31989/ffhd.v15i8.1705
18. Han L, Wang H, Cao J, Li Y, Jin X, He C, *et al*. Inhibition mechanism of α-glucosidase inhibitors screened from Tartary buckwheat and synergistic effect with acarbose. *Food Chem*. 2023; 420:136102.
DOI: http://doi.org/10.1016/j.foodchem.2023.136102.
19. Dang D, Li D, Xu S, He F, Xia X, Xu S. Pharmacological properties of Humulus scandens: Insights from the TCMSP database and existing understanding. *Pharmacol Res - Mod Chin Med*. 2024;13: 100518.
DOI: http://doi.org/ 10.1016/J.PRMCM.2024.100518.
20. Consortium U. UniProt: The universal protein knowledgebase in 2023. *Nucleic Acids Res*. 2023;51(D1):D523-D531.
DOI: http://doi.org/10.1093/nar/gkac1052.
21. Davis P A, Wiegers C T, Johnson J R, Sciaky D, Wiegers J, Mattingly J C. Comparative toxicogenomics database (CTD): Update 2023. *Nucleic Acids Res*. 2023;51(D1):D1257-D1262.
DOI: http://doi.org/10.1093/nar/gkac833.
22. Su R, Chen Y, Yi C, Zhu Y, Feng Q, Guo Y, *et al*. Integrating data mining, network pharmacology, molecular docking and molecular dynamics simulation to explore medication regularity and mechanism of Corydalis yanhusuo, *Chin Herb Med*. 2025;56(14):5135–5150. (in Chinese)
DOI: http://doi.org/10.7501/j.issn.0253-2670.2025.14.018.
23. Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, *et al*. The STRING database in 2025: protein networks with directionality of regulation. *Nucleic Acids Res*. 2025;53(D1):D730–D737.
DOI: http://doi.org/10.1093/nar/gkae1113.
24. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi H A, *et al*. Metascape provides a biologist-oriented resource for the analysis of systemslevel datasets. *Nat Commun*. 2019;10(1):1523.
DOI: http://doi.org/10.1038/s41467-019-09234-6.
25. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, *et al*. PubChem 2025 update. *Nucleic Acids Res*. 2025;53(D1):D1516–D1525.
DOI: http://doi.org/10.1093/nar/gkae1059.
26. Flachsenberg F, Ehrt C, Gutermuth T, Rarey M. Redocking the PDB. J. Chem. Inf. Model., 2024;64(1):219–237.
DOI: http://doi.org/10.1021/acs.jcim.3c01573.
27. Liu Y, Cao Y. Protein-Ligand Blind Docking Using CB-Dock2. *Methods Mol Biol*. 2024;2714: 113-125.
DOI: http://doi.org/ 10.1007/978-1-0716-3441-7_6.
28. Shannon P, Markiel A, Ozier O, Baliga S N, Wang T J, Ramage D, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–504.
DOI: http://doi.org/10.1101/gr.1239303.
29. Zhou S, Zhang Z, Li S, Liu M, Zhang Z, Wang N, *et al*. Characterization and efficacy of α-glucosidase inhibitory peptides from enzymatically hydrolyzed Peanut meal. *Food Res Int*. 2025; 218:116864.
DOI: http://doi.org/10.1016/j.foodres.2025.116864.
30. Hsin K, Ghosh S, Kitano H. Combining machine learning systems and multiple docking simulation packages to improve docking prediction reliability for network pharmacology. *PLoS ONE*. 2013;8(12):e83922.
DOI: http://doi.org/10.1371/journal.pone.0083922.
31. Singh N, Jogana A Z, Khanna S. Theoretical and experimental approaches to thiosemicarbazone: spectroscopy, molecular docking and ADMET studies. *Phosphorus, Sulfur, Silicon Relat Elem*. 2025;200(9):694–705.
DOI: http://doi.org/10.1080/03014460.2024.239876.
32. Cao X, Shi K, Xu Y, Zhang P, Zhang H, Pan S. Integrated metabolomics and network pharmacology to reveal antioxidant mechanisms and potential pharmacological ingredients of citrus herbs. *Food Res Int*. 2023;174(P1):113514.

DOI: http://doi.org/10.1016/j.foodres.2023.113514.

33. Pasdaran A, Hamedi A, Shiehzadeh S, Hamedi A. A review of citrus plants as functional foods and dietary supplements for human health, with an emphasis on meta-analyses, clinical trials, and their chemical composition. *Clin Nutr ESPEN*. 2023;54:311–336.
DOI: http://doi.org/10.1016/j.clnesp.2023.02.001.
34. Wang X, Xie Y, Yu J, Chen Y, Tian Y, Wang Z, et al. On-site analysis and rapid identification of citrus herbs by miniature mass spectrometry and machine learning. Rapid Commun Mass Spectrom. 2023;174(P1):113514.
DOI: http://doi.org/10.1016/J.FOODRES.2023.113514.
35. Xiao Y, Shen J, Zhan J, Guo L, Ho C T, Li S. Antiglycating effects of citrus flavonoids and associated mechanisms. *Food Sci Hum Wellness*. 2024;13(5):2363–2372.
DOI: http://doi.org/10.1016/j.fshw.2024.04.018.
36. Salem E, Ta A, Fueger P T, Wang P H. 380-P: Dual roles of renal tubular mitochondrial AKT1 in improving whole body glucose metabolism and protecting against diabetic nephropathy. *Diabetes*. 2025; 74:1.
DOI: http://doi.org/10.2337/db25-380-P.
37. Yu S, Liu W. Protective effects of Salidroside against ferroptosis through PPARG-dependent mechanism in diabetes-related cognitive impairment. *Exp Neurol*. 2025;390:115261.
DOI: http://doi.org/10.1016/j.expneurol.2025.115261.
38. Fang T, Wang J, Sun S, Deng X, Xue M, Han F, *et al*. JinLiDa granules alleviates cardiac hypertrophy and inflammation in diabetic cardiomyopathy by regulating TP53. *Phytomedicine*. 2024;130:155659.
DOI: http://doi.org/10.1016/j.phymed.2024.155659.
39. Yi W, Si Y, Yi S, Yuan W, Zheng W, Ping S, *et al*. Resveratrol protects müller cells against ferroptosis in the early stage of diabetic retinopathy by regulating the Nrf2/GPx4/PTGS2 pay. *Mol Neurobiol*. 2024;62(3):1–16.
DOI: http://doi.org/10.1007/s12035-024-04496-8.