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Network pharmacology-based exploration of gut microbiota-derived metabolites for type-2 diabetes

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ABSTRACT Probiotics confer health benefits and have been investigated for their potential therapeutic properties in type-2 diabetes (T2D) treatment. This study employs a network pharmacology approach to explore gut microbiota-derived metabolites that potentially alleviate T2D. Several strains and species of gut microbiota were identified that may produce metabolites with therapeutic potential for T2D. Interestingly, quercetin produced by *Bacteroides uniformis* and daidzein produced by *Bifidobacterium adolescentis* and *Bifidobacterium breve* have been studied for their antidiabetic effects. Using a network pharmacology approach, it was found that quercetin may target AKT1 and EGFR, critical proteins involved in insulin signaling pathways related to T2D. Additionally, 10-oxo-11-octadecenoic acid produced by *Lactobacillus plantarum* and 10-keto-12Z-octadecenoic acid produced by *Lactobacillus paracasei* were found to target PPARG, a gene regulating insulin signaling. These findings were further validated by the molecular docking analysis, which showed suitable to satisfactory binding strengths.

KEYWORDS Diabetes; Gut microbiota; Insulin signaling; Network pharmacology

1. Introduction

Type-2 diabetes mellitus (T2D) is a globally prevalent metabolic disease characterized by highly elevated fasting blood glucose (FBG), also known as hyperglycemia, primarily caused by pancreatic beta-cell dysfunction and insulin resistance (IR). Various factors, including ethnicity, genetic predisposition, obesity, sedentary lifestyle, and unhealthy diet, influence this disruption in glucose homeostasis (Galicia-García et al. 2020). Current treatments for T2D involve a multifaceted approach, encompassing lifestyle modifications and pharmacological interventions (Manaf et al. 2016). Lifestyle changes include dietary and exercise regimens to improve glycemic control. Pharmacological intervention may consist of the use of biguanides, sulfonylureas, alpha-glucosidase inhibitors, thiazolidinediones (TZDs), dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter-2 (SGLT-2) inhibitors, glucagon-like peptide-1 receptor agonists (GLP-1 RAs), as well as insulin treatment. Each pharmacological intervention operates through distinct mechanisms, such as reducing blood glucose levels, enhancing insulin sensitivity, or stimulating insulin production by pancreatic beta-cells (Marín-Peñalver et al. 2016). Despite the availability of various drug treatments, there remains a significant need for novel therapeutic agents to prevent and manage diabetes effectively and to overcome the limitations of current T2D treatment (Tjokroprawiro et al. 2016; Tan et al. 2023a; Kurniawan et al. 2024).

In recent years, human gut microbiota has emerged as a key player in human metabolic health. The human gut comprises trillions of microbial inhabitants, including bacteria, archaea, fungi, and viruses (Jandhyala et al. 2015). These microbes are estimated to encode more than 3 million genes responsible for producing a vast array of metabolites (Rinninella et al. 2019). The production of metabolites, including short-chain fatty acids (SCFAs), fosters the gut microbiota to work with the host to maintain immune homeostasis (Rooks and Garrett 2016). The gut microbiota also facilitates essential nutrient extraction from food, such as generating SCFAs through carbohydrate fermentation (Carding et al. 2015).

The gut microbiota predominantly comprises Firmicutes and Bacteroidetes, two bacterial phyla comprising around 90% of the gut inhabitants (Rinninella et al. 2019). There is growing recognition of the relationship between gut microbiota composition and human health, supported by several studies investigating the differences in gut mi-

crobiota composition between healthy individuals and individuals with various metabolic diseases, such as T2D and obesity (Larsen et al. 2010; Kasai et al. 2015; Crovesy et al. 2020; Li et al. 2020a; Widjaja et al. 2025).

Gut microbiota-derived metabolites offer promising leads for identifying therapeutic targets in metabolic disorders, including T2D, paving the way for the development of innovative treatments (Agus et al. 2021). Probiotics are live organisms that can confer health benefits to the host when administered in adequate doses (Wulandari et al. 2016). Recently, probiotics have been investigated for their potential therapeutic properties in treating T2D. Moreover, previous studies have indicated the potential of probiotics in reducing FBG and glycosylated hemoglobin (HbA1c) and improving insulin sensitivity and glycemic control (Tonucci et al. 2017; Tao et al. 2020). However, the specific mechanisms of the gut microbiota and their corresponding metabolites and molecular targets have yet to be elucidated adequately in the intricate gut microbiome system (Oh et al. 2022a,b). Hence, we attempt to address this knowledge gap by leveraging a network pharmacology approach to integrate microbiota, metabolites, target proteins, and signaling pathways, thereby identifying potential therapeutic connections between gut microbiota-derived metabolites and T2D-related targets. This study aims to establish a systematic framework for identifying novel microbiota-target interactions that can inform future drug development for T2D.

2. Materials and Methods

2.1. Collection of gut microbiota metabolites

Metabolites derived from the human gut microbiota were collected from the gutMGene database v1.0 (https://bio-computing.hrbmu.edu.cn/gutmgene/#/home) and downloaded them from the downloads section on the gutMGene website (Cheng et al. 2022). We acquired the molecule name, PubChem CID, and canonical Simplified Molecular Input Line Entry System (SMILES) of the identified metabolites from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al. 2023).

2.2. Mining and screening of target proteins

Potential target proteins of human gut microbiota-derived metabolites were retrieved using the Similarity Ensemble Approach (https://sea.bkslab.org/) (Keiser et al. 2007) and SwissTargetPrediction (http://www.swisstargetprediction.ch/) (Daina et al. 2019) by submitting the canonical SMILES of each metabolite. The target proteins were further screened based on two criteria: derived from humans (*Homo sapiens*) and had a Tanimoto coefficient (TC) of at least 0.5. The retained target proteins were standardized using the UniProt database (https://www.uniprot.org/) (The UniProt Consortium 2023) and then screened for duplicates to eliminate redundant entries.

We fetched T2D target proteins from the GeneCards database v5.19 (https://www.genecards.org) (Stelzer et al. 2016; Safran et al. 2021) and Discovery Platform for the GENomic and Environmental T v24 (DisGeNet, https://www.disgenet.org/) (Piñero et al. 2015, 2017, 2021) by searching for the term "type-2 diabetes". We standardized all T2D-related target proteins using the UniProt database and removed the duplicates. The common target proteins, which are proteins related to gut microbiota metabolites and T2D, were identified.

2.3. Construction of protein-protein interaction (PPI) networks

The stringApp on Cytoscape v3.10.2 (https://cytoscape.org/) (Doncheva et al. 2019) was employed to construct individual PPI networks of gut microbiota metabolites-derived target proteins and T2D target proteins. We set the species to *Homo sapiens* (human) and the confidence level to 0.700. We merged the two PPI networks and then analyzed them using CytoNCA tools (Tang et al. 2015) to assess network topology parameters, such as degree centrality (DC), eigenvector centrality (EC), betweenness centrality (BC), and closeness centrality (CC). We selected target proteins with DC values surpassing twice the median of all DC values as the potential target proteins. We selected metabolites associated with a large number of those potential target proteins as the crucial metabolites. Then, we constructed the potential target proteins into a PPI network and reanalyzed them using CytoNCA. We subsequently identified the crucial targets by selecting those with DC, EC, BC, and CC values higher than the median of each respective parameter. CytoNCA analysis was repeated twice, and only protein targets with consistently high centrality values were retained, ensuring robust selection of proteins likely to have strong and functionally relevant interactions.

2.4. GOs and KEGG pathway enrichment analysis

Utilizing Enrichr (https://maayanlab.cloud/Enrichr/) (Kuleshov et al. 2016; Xie et al. 2021), the crucial targets were analyzed for enrichment in GO biological processes (BPs), molecular functions (MFs), and cellular components (CCs), as well as KEGG 2021 Human (Kanehisa et al. 2023). The significant pathways and GO terms ($p \leq 0.05$) were selected and subsequently visualized using SRplot (https://bioinformatics.com.cn/) (Tang et al. 2023).

2.5. Construction of microbiota-metabolites-targets-signaling pathway (MMTS) network

Three individual networks were constructed on Cytoscape v3.10.2 by importing three text files (.txt) containing: (1) the microbiota and their associated metabolites, (2) the metabolites and their crucial target proteins, and (3) the essential target proteins and their associated signaling pathways. The three networks were constructed into a union using the merge tool. The union represents the MMTS network.

2.6. Molecular docking

The CB-Dock web server (http://cao.labshare.cn/cb-dock/) (Liu et al. 2020) were utilized to analyze molecular docking. The 3D structures of the metabolites and target proteins were retrieved from PubChem and RCSB Protein Data Bank (https://www.rcsb.org/) (Rose et al. 2016) respectively. The University of California, San Francisco (UCSF) Chimera alpha v.1.18 (https://www.cgl.ucsf.edu/chimera/download.html) (Pettersen et al. 2004) removed any non-protein residues attached to the retrieved 3D protein structures. The 3D structures of the metabolites and the proteins were submitted to the CB-Dock web server as the ligand and the protein, respectively, to collect information regarding the Vina score, predicted binding regions, and binding mode images. The Vina score reflects the binding strength, which is further classified into three categories: potential (affinity of < −4.25 kcal/mol), good (affinity of < −5.00 kcal/mol), and satisfactory (affinity of < −7.00 kcal/mol) (Liu et al. 2021). This classification serves as a foundation for identifying favorable binding among the complexes.

3. Results and Discussion

3.1. Gut microbiota-derived metabolites

Information about the gut microbiota and related metabolites was retrieved from the gutMGene database, revealing 334 microbes (Table S1) and 208 distinct metabolites (Table S2). The list of microbes encompassed several species and strains of *Bifidobacterium*, *Bacteroides*, and *Lactobacillus*, among others. These microbes were reported to produce, daidzein, quercetin, 10-keto-12Z-octadecenoic acid, and other metabolites. Of all metabolites, 23 were derived from unknown species/strains of the gut microbiota. A comprehensive list of microbes and their corresponding metabolites is provided in Table S1.

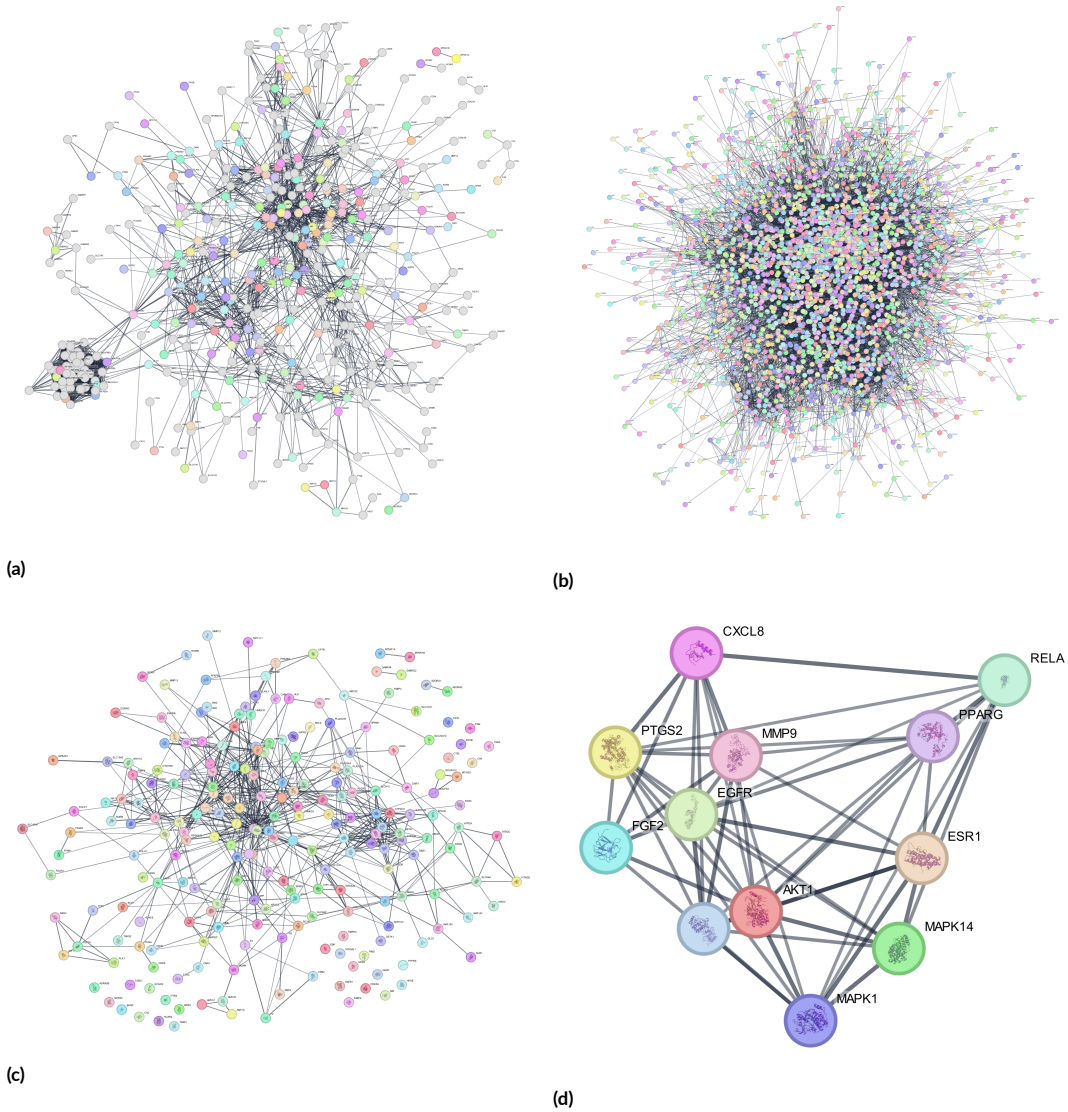


FIGURE 1 PPI networks were constructed in this study. (a) PPI network of gut microbiota metabolites target proteins; (b) PPI network of T2D target proteins; (c) PPI network of common target proteins; (d) PPI network of crucial target proteins.

3.2. Target proteins related to T2D and gut microbiota-derived metabolites

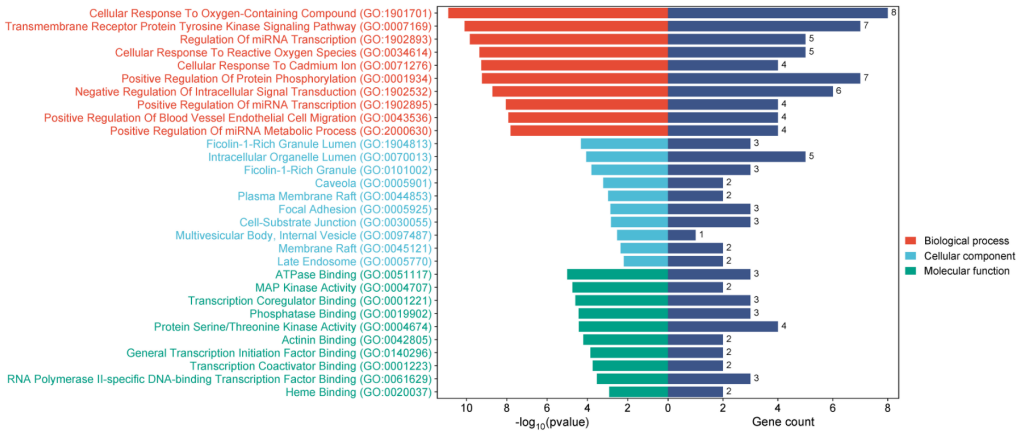
The metabolites were subjected to target protein prediction using the Similarity Ensemble Approach and SwissTargetPrediction, resulting in 464 unique target proteins (Tables S3 and S4). Mining T2D target proteins from GeneCards and DisGeNet databases yielded 2,828 unique target proteins (Table S5). The typical target proteins were identified (Table S6).

3.3. PPI networks

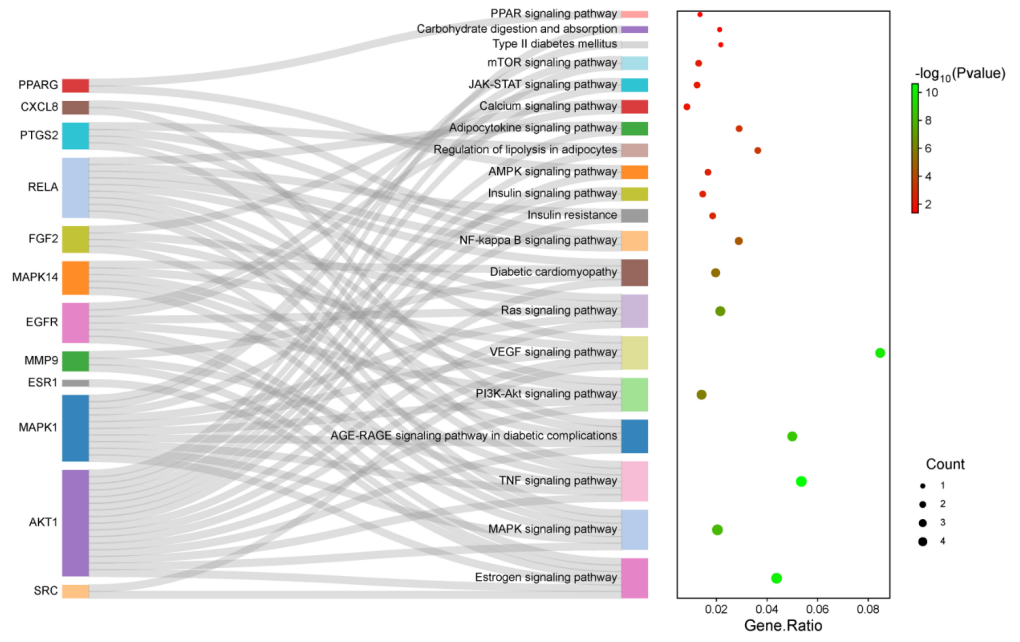
The PPI network of gut microbiota-derived metabolites target proteins (Figure 1a) consisted of 463 nodes and 2,586 edges, and the PPI network of T2D target proteins (Figure 1b) consisted of 2,796 nodes and 22,858 edges. We constructed the intersection of both networks, resulting in the PPI network of common target proteins (Figure 1c), which consisted of 223 nodes and 649 edges. Network topology analysis of the typical target proteins yielded 12 crucial target proteins (Tables S7, S8, and S9) and revealed the top crucial metabolites: quercetin, myricetin, chrysin, and apigenin (Table S10). We constructed the crucial target proteins into the final PPI network (Figure 1d).

3.4. GOs and KEGG pathway enrichment analysis

A total of 12 crucial targets were subjected to functional annotation using the Enrichr web server, resulting in 614 BP terms, 81 MF terms, 27 CC terms, and 142 KEGG pathways ($p < 0.05$) (Tables S11 and S12). Figure 2a shows the top 10 BPs, MFs, CCs, and KEGG pathways related to T2D. The top 20 KEGG pathways were also identified (Table S13 and Figure 2b) and subjected to MMTS network construction.



(a)



(b)

FIGURE 2 Enrichment analysis of the crucial targets. (a) A bar graph of the top 10 enriched BPs, MFs, and CCs. (b) A Sankey diagram with a bubble plot illustrating the top 20 KEGG pathways and their related genes.

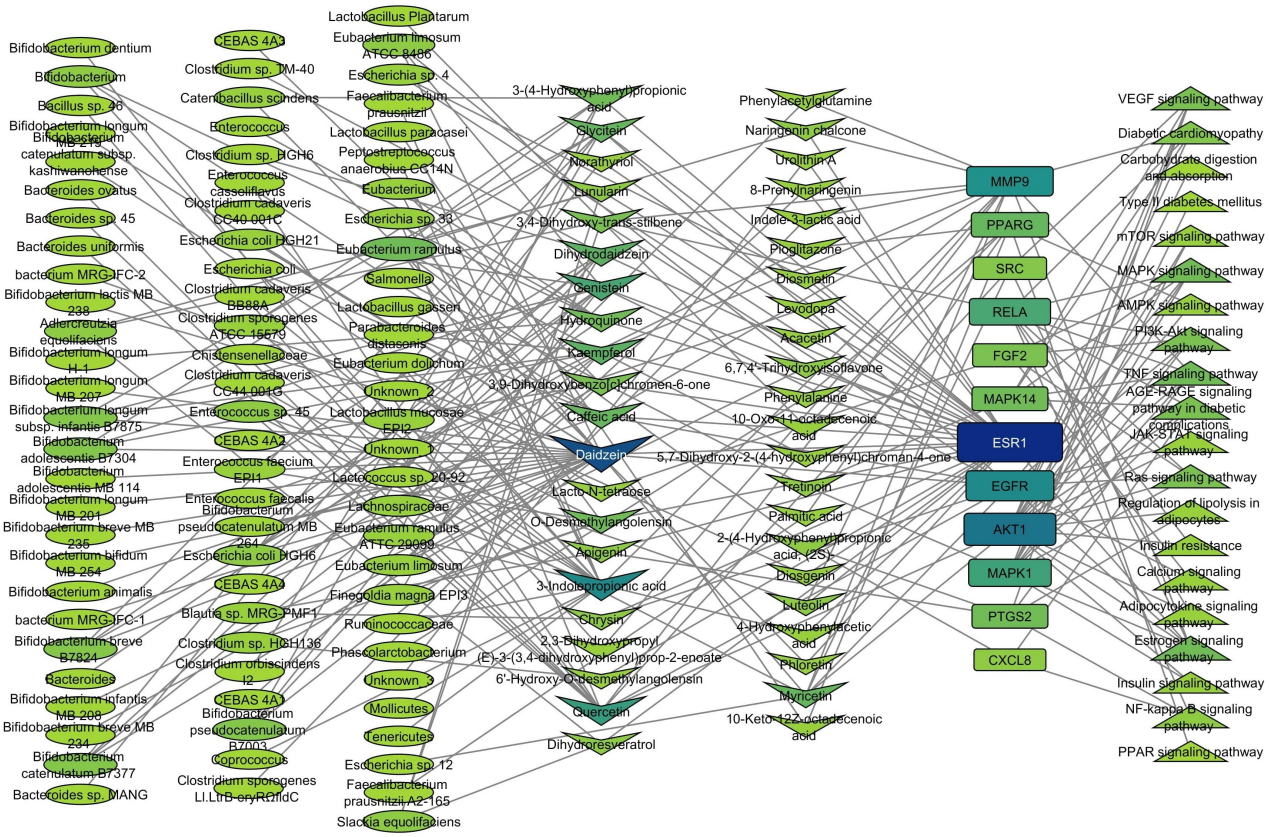


FIGURE 3 MMTS network depicting the interactions between gut microbiota-associated crucial metabolites, crucial target proteins, and signaling pathways about T2D.

TABLE 1 The binding energies of the top metabolites and targets associated with T2D and the gut microbiota.

Protein	PDB ID	Ligand	Vina Score (kcal/mol)	Cavity Size	Docking Center			Size of Predicted Cavity		
					x	y	z	x	y	z
AKT1	1UNQ	Quercetin	−6.8	847	28	17	5	21	21	21
		Myricetin	−7.1	229	25	8	18	21	21	21
		Chrysin	−6.5	847	28	17	5	20	20	20
		Apigenin	−6.6	847	28	17	5	21	21	21
		Daidzein	−6.8	847	28	17	5	21	21	21
EGFR	1IVO	Quercetin	−8	5897	99	92	45	34	31	21
		Myricetin	−8.1	5897	99	92	45	34	31	21
		Chrysin	−7.5	3468	129	43	40	21	35	21
		Apigenin	−7.5	3468	129	43	40	21	35	21
		Daidzein	−6.9	2615	60	24	75	31	21	29
PTGS2	5F19	Quercetin	−7.2	6299	10	27	54	21	34	35
		Myricetin	−7.4	6299	10	27	54	21	34	35
		Chrysin	−6.9	6035	40	49	20	31	35	30
		Apigenin	−6.9	6035	40	49	20	31	35	30
		Daidzein	−6.6	5452	25	43	50	32	21	21
PPARG	8B8W	Quercetin	−7.6	544	24	14	27	21	21	21
		Myricetin	−7.6	544	24	14	27	21	21	21
		Chrysin	−7.8	544	24	14	27	20	20	20
		Apigenin	−7.9	544	24	14	27	21	21	21
		Daidzein	−7.7	311	12	14	25	21	21	21

3.5. MMTS network

The MMTS network consisted of 83 species and strains of the gut microbiota, 43 metabolites, 12 crucial target proteins, and the selected 20 KEGG pathways. Circular nodes represent the gut microbiota, V-shaped nodes for the metabolites, rectangular nodes for the crucial target proteins, and triangular nodes for the signaling pathways (Figure 3). A bigger node size and a darker node color indicate a higher DC value.

3.6. Molecular docking

The crucial compounds as ligands and the crucial targets as proteins were submitted. Daidzein was included as one of the compounds due to its association with PPARG, a gene regulating insulin signaling, and previous findings regarding its potential effects on T2D. The molecular docking analysis gathered information about the Vina scores and binding regions (Table 1) and yielded the docking mode diagram (Figure 4). The best result was EGFR–myricetin, which exhibited the lowest Vina scores of all docking attempts, indicating the most favorable binding interaction.

3.7. Discussion

In this study, network topology analysis identified four metabolites as the most crucial: quercetin, myricetin, chrysin, and apigenin. Quercetin is a naturally occurring flavonol that possesses a myriad of potential bioactivities, such as anticarcinogenic, anti-inflammatory, antiviral, and antioxidant activities (Li et al. 2016b). Several previous studies have indicated the possible role of quercetin in carbohydrate and glucose metabolism. For instance, the findings of Oyedemi et al. (2020) implicated the role of quercetin in glucose homeostasis, which demonstrated that oral administration of quercetin in diabetic rats could reduce blood glucose levels, reduce HbA1c levels, and increase glycogen storage—moreover, a reduced risk of T2D associated with a higher intake of foods rich in quercetin (Knekt et al. 2002).

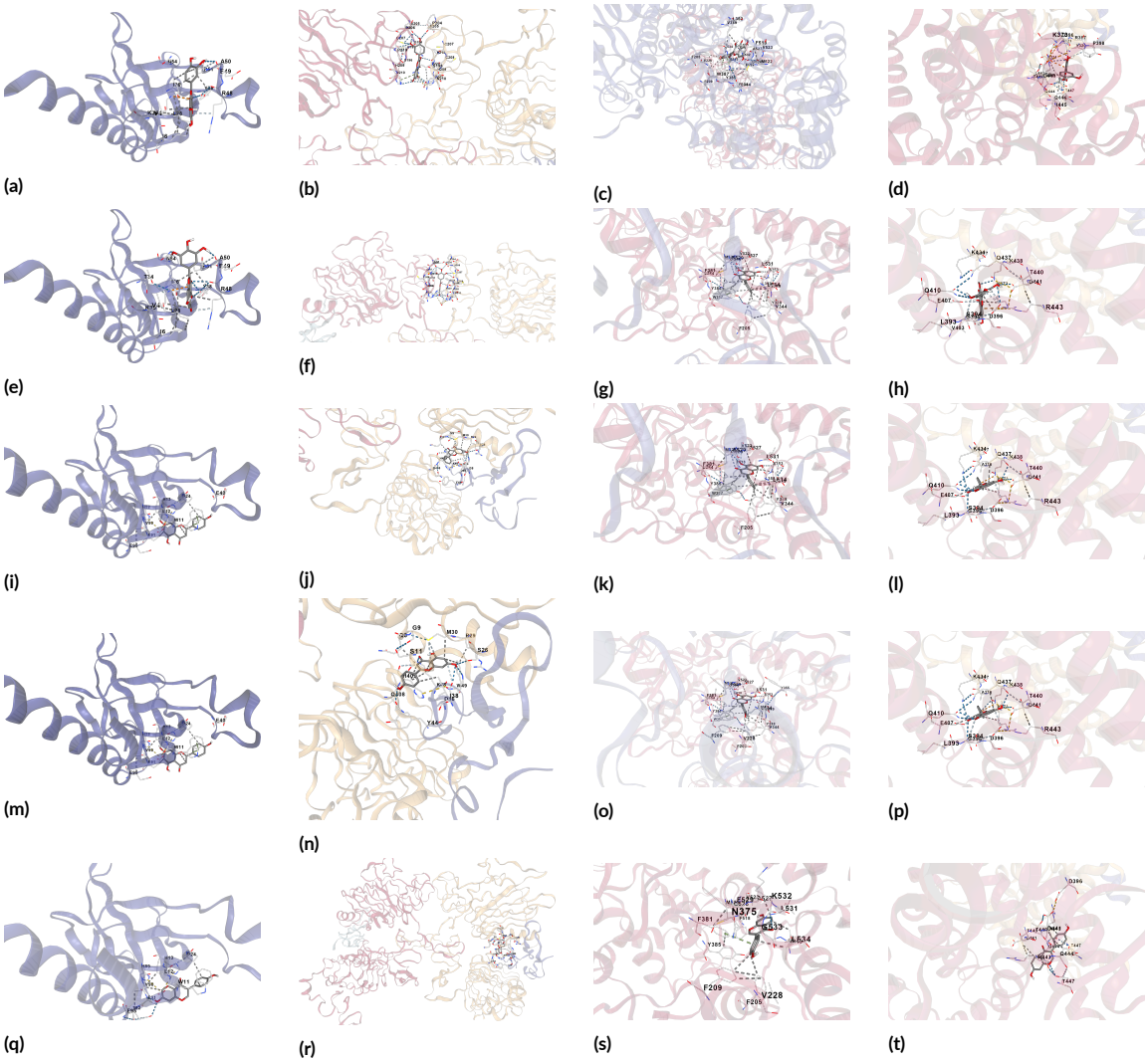


FIGURE 4 Docking modes of the top crucial metabolites and crucial targets associated with T2D. (a) Quercetin–AKT1; (b) Quercetin–EGFR; (c) Quercetin–PTGS2; (d) Quercetin–PPARG; (e) Myricetin–AKT1; (f) Myricetin–EGFR; (g) Myricetin–PTGS2; (h) Myricetin–PPARG; (i) Chrysin–AKT1; (j) Chrysin–EGFR; (k) Chrysin–PTGS2; (l) Chrysin–PPARG; (m) Apigenin–AKT1; (n) Apigenin–EGFR; (o) Apigenin–PTGS2; (p) Apigenin–PPARG; (q) Daidzein–AKT1; (r) Daidzein–EGFR; (s) Daidzein–PTGS2; (t) Daidzein–PPARG.

TABLE 2 Crucial metabolites and their associated members of the gut microbiota.

Metabolite	Producers
Quercetin	*Bacillus* sp. 46
	Bacteroides ovatus
	Bacteroides sp. 45
	Bacteroides uniformis
	Bifidobacterium dentium
	Enterococcus casseliflavus
	Enterococcus sp. 45
	Escherichia sp. 33
	Enterococcus sp. 45
Myricetin	*Escherichia* sp. 12
	Escherichia sp. 33
Chrysin	*Blautia* sp. MRG-PMF1
Apigenin	*Blautia* sp. MRG-PMF1
Luteolin	*Enterococcus* sp. 45
Kaempferol	*Bifidobacterium pseudocatenulatum* B7003
	Bifidobacterium longum subsp. *infantis* B7875
	Bifidobacterium adolescentis B7304
	Bifidobacterium catenulatum B7377
	Bifidobacterium breve B7824
Acacetin	*Escherichia* sp. 4
Baicalein	Unknown
Diosmetin	*Escherichia* sp. 4
Daidzein	*Bifidobacterium adolescentis* MB 114
	Bifidobacterium bifidum MB 254
	Bifidobacterium breve MB 234
	Bifidobacterium breve MB 235
	Bifidobacterium infantis MB 208
	Bifidobacterium lactis MB 238
	Bifidobacterium longum MB 201
	Bifidobacterium longum MB 207
	Bifidobacterium longum MB 219
	Bifidobacterium pseudocatenulatum MB 264
	bacterium MRG-IFC-1
	bacterium MRG-IFC-2
	Escherichia coli HGH21
	Escherichia coli HGH6
	Eubacterium limosum ATCC 8486
	Catenibacillus scindens
	Bifidobacterium pseudocatenulatum B7003
	Bifidobacterium longum subsp. *infantis* B7875
	Bifidobacterium adolescentis B7304
	Bifidobacterium catenulatum B7377
	Bifidobacterium breve B7824

Myricetin is also a flavonol known for its iron-chelating, antioxidant, anti-inflammatory, and anticancer activities (Knekt et al. 2002; Semwal et al. 2016). Myricetin inhibits the cyclin-dependent kinase 5 (CDK5) activation, which has implications for mitochondrial dysfunction and beta-cell apoptosis. Hence, myricetin's inhibition of CDK5 activation may exert protective effects on pancreatic beta-cells (Karunakaran et al. 2019). Like quercetin, a decreased risk of T2D correlated with a higher myricetin intake (Knekt et al. 2002).

Chrysin is a flavone abundantly found in many plant extracts. Knowledge regarding the therapeutic effects of chrysin remains limited due to its low bioavailability. However, a study by Ramírez-Espinosa et al. (2017) found that chrysin significantly reduces pro-inflammatory cytokines and has notable antihyperglycemic and antidiabetic effects in diabetic rats. Acute and sub-acute treatments (50 mg/kg) also reduced triglyceride levels and diminished IL-1β and TNF-α. These findings suggest that chrysin could offer similar benefits to metformin by lowering glucose and triglyceride levels and inhibiting pro-inflammatory cytokines associated with diabetes complications.

Apigenin is a metabolite that also belongs to the flavone subclass of flavonoids. Numerous studies have indicated the potential antidiabetic activity of apigenin. Ren et al. (2016) reported attenuating FBG levels and insulin resistance index (IRI) following a 6-week apigenin administration at 50 mg/kg and 100 mg/kg. Panda and Kar (2007) also reported the potential T2D ameliorating effects of apigenin, demonstrated through the reduction in blood glucose level, increase in insulin level, and normalization of superoxide dismutase (SOD) and catalase (CAT) activity. This study also discovered other metabolites that appear to be quite crucial, such as luteolin, kaempferol, acacetin, baicalein, diosmetin, and daidzein (refer to Table S10). These metabolites are biosynthesized by several species and strains of the gut microbiota, as presented in Table 2 (refer to Table S1).

Several species and strains produce quercetin, including *B. uniformis*, considered one of the next-generation probiotic candidates (Vallianou et al. 2023). Previously, Wu and Park (2022) reported that *B. uniformis* negatively correlates with T2D risk in Asian cohorts. Park et al. (2023) also found that the abundance of *B. uniformis* in American T2D patients was lower than that of healthy individuals, further supporting the findings of Wu and Park (2022) regardless of the difference in cohorts. Furthermore, Li et al. (2020b) found that an increased abundance of *B. uniformis* could down-regulate inflammatory cytokines, attenuating low-grade inflammation and enhancing insulin sensitivity. Moreover, the role of *B. uniformis* has been implicated in glucose metabolism since it regulates plasma insulin and glucagon-like peptides via the indirect regulation of gluconeogenesis genes like peroxisome proliferator-activated receptor gamma (PPARγ).

Notably, daidzein appears to be produced by the most significant number of species and strains (Table 2). Daidzein is an isoflavone that has antidiabetic or antihyperglycemic effects (Das et al. 2018). Park et al. (2006) demonstrated that daidzein supplementation could attenuate blood glucose levels by increasing hepatic glucokinase (GK) and decreasing glucose-6-phosphatase (G6Pase) ac-

tivity in db/db mice. The *in vitro* and *in vivo* findings of Cheong et al. (2014) also suggested that daidzein may mediate its antihyperglycemic effect by AMPK activation via GLUT4 translocation to the plasma membrane of muscle cells, where glucose uptake occurs. In plants, daidzein mainly exists in its inactive form, daidzin (Das et al. 2018).

Interestingly, Raimondi et al. (2009) reported that the ability of several *Bifidobacterium* strains to convert daidzin to daidzein supports the findings of this present study (Table 2). Previously, Sedighi et al. (2017) found that *Bifidobacterium* was significantly more abundant in healthy cohorts compared to T2D cohorts. Furthermore, supplementation of several *Bifidobacterium* strains has shown potential in enhancing insulin sensitivity, which may help in T2D treatment (Zhang et al. 2020). Chaiyasut et al. (2023) also indicated that *B. breve* supplementation significantly decreased creatinine, low-density lipoprotein, triglycerides, and HbA1c levels, suggesting it could prevent deterioration in T2D patients.

Network topology analysis also revealed crucial targets associated with T2D, such as EGFR, AKT1, PTGS2, and PPARG (Table S9). Epidermal growth factor receptor (EGFR) belongs to the ErbB family of tyrosine kinase receptors. EGFR undergoes autophosphorylation upon binding to its ligand, thereby triggering downstream signaling pathways that play a crucial role in regulating cellular processes, such as proliferation, differentiation, and survival (Sigismund et al. 2018). Li et al. (2018) demonstrated that EGFR inhibition improved diabetic nephropathy (DN) and insulin resistance in db/db mice with endothelial nitric oxide knockout. They also proposed several mechanisms by which EGFR inhibition benefits diabetic nephropathy. Firstly, EGFR inhibition directly protects against DN by inhibiting the infiltration of immune cells and oxidative stress in the kidneys, thereby reducing the expression of fibrotic and profibrotic components. Secondly, EGFR inhibition indirectly benefits DN by enhancing islet autophagy, which preserves pancreatic beta-cell function and improves metabolic status. Thirdly, EGFR inhibition increases circulating levels of adiponectin, an adipokine with insulin-sensitizing, anti-inflammatory, and kidney-protective effects. This study revealed that quercetin and daidzein target EGFR, which supports the possibility that these metabolites may play a role in ameliorating T2D.

AKT1 is a member of the Akt kinase family, which plays a role in various biological processes, including cell proliferation, growth, survival, and metabolism (Truebestein et al. 2021). The enrichment analysis shows that AKT1 is involved in the PI3K-Akt signaling pathway. When insulin binds to its receptor, the receptor undergoes phosphorylation and activates insulin receptor substrate-1 (IRS-1), allowing IRS-1 to initiate several signaling pathways like the PI3K-Akt pathway. Within this pathway, the phosphoinositide 3-kinase (PI3K) enzyme catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2 or PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P3 or PIP3). PIP3 then binds to Akt, triggering the phosphorylation of Akt by PDK1. Phosphorylated Akt may phosphorylate other targets that eventuate in GLUT4 translocation to the plasma membrane (Tjandrawinata et al. 2012; Tan et al. 2023b). Overexpression or enhanced activity of Akt in T2D increases glucose uptake in skeletal muscles, thereby maintaining euglycemia. Conversely, attenuated Akt signaling is associated with insulin resistance in metabolic tissues, leading to T2D. Additionally, Akt is crucial for insulin-mediated glucose uptake in the liver and for suppressing glucagon secretion from pancreatic α-cells, thereby reducing hepatic glucose production (Alwhaibi et al. 2019).

Based on the enrichment analysis, both EGFR and AKT1 were involved in the PI3K-Akt pathway (Figure 5), which plays a role in cellular processes, influencing cell proliferation, differentiation, metabolic regulation, and cytoskeletal reorganization. This pathway also regulates apoptosis and cancer cell survival, associated with various diseases, including obesity, diabetes, and cancer (Huang et al. 2018). Furthermore, the enrichment analysis also showed the involvement of EGFR and AKT1 in Ras and MAPK signaling pathways, both of which are involved in the upstream insulin signaling pathway (Figure 5) (Świderska et al. 2020). However, Akt2 appears to play a more critical role in glucose metabolism than Akt1, although cells ubiquitously express Akt1 (Miao et al. 2022).

PTGS2 gene encodes the proinflammatory enzyme cyclooxygenase-2 (COX-2) (Markosyan et al. 2019). This protein is expressed in response to inflammatory stimuli, leading to prostaglandin (PG) release, thereby contributing to inflammation (Hellmann et al. 2015). COX-2-mediated visceral fat inflammation develops insulin resistance and fatty liver in high-fat-induced obese rats (Hsieh et al. 2009). This study also identified PPARG as one of the insulin signaling-regulating genes. It encodes the PPARγ, a nuclear receptor that regulates carbohydrate and lipid metabolism and fat tissue differentiation. Adipose tissue primarily expresses PPARγ, where it modulates the expression of adiponectin (Tjandrawinata 2016; Permadi et al. 2021). PPARG appears to be a crucial target in T2D treatment as its activation enhances insulin sensitivity and upregulates gene expression in glucose uptake (Astuti et al. 2022).

This study identified several metabolites, such as pioglitazone, 10-oxo-11-octadecenoic acid, phenylalanine, palmitic acid, and 10-keto-12Z-octadecenoic acid, which target the PPARG gene. The metabolites are produced by an unknown strain, *Lactobacillus plantarum*, *Phascolarctobacterium*, *Faecalibacterium prausnitzii* A2-165, and *Lactobacillus paracasei*, respectively. Several researchers have previously studied the antidiabetic effects of *L. plantarum* and *L. paracasei* strains. Li et al. (2016a) showed that *L. plantarum* CCFM0236 exhibited potential hypoglycemic effects by alleviating mice's insulin resistance and systemic inflammation. Bejar et al. (2013) demonstrated that *L. plantarum* TN627 exerted potential antidiabetic effects, as shown in the decreased blood glucose level and enhanced glucose tolerance in adult rats. Zeng

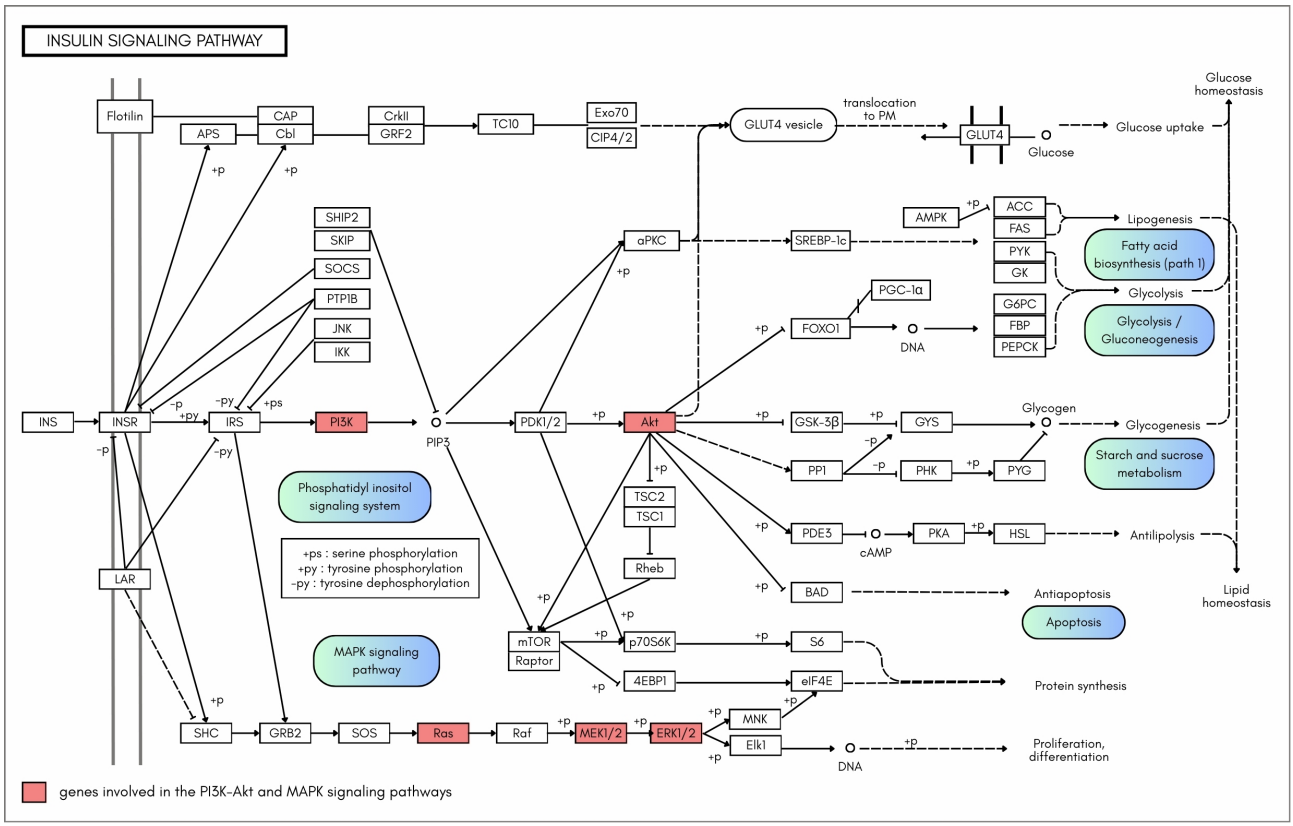


FIGURE 5 Insulin signaling pathway (KEGG: 04910).

et al. (2019) indicated that *L. paracasei* NL41 may prevent the development of a high-fat diet/streptozotocin-induced T2D (HFD/STZ-T2D) by reducing insulin resistance and oxidative stress levels while preserving beta-cell function. Those previous studies led the authors to postulate that *L. plantarum* and *L. paracasei* may serve as promising probiotic candidates. However, the prior studies were conducted using different strains and may not represent identical outcomes. Hence, the potential of *L. plantarum* and *L. paracasei* strains as antidiabetic supplements warrants further investigation to assess their safety and effectiveness in mitigating the symptoms of T2D.

The enrichment analysis revealed several enriched BP terms that may elucidate the mechanisms by which gut microbiota-derived metabolites may ameliorate T2D. The categories for the enriched BP terms are as follows: insulin, inflammation, glucose metabolism, and lipid metabolism. The BP terms related to insulin include response to insulin (GO:0032868), cellular response to insulin stimulus (GO:0032869), and regulation of cellular response to insulin stimulus (GO:1900076). Insulin maintains glucose homeostasis in skeletal muscles, adipose tissue, and the liver (Astuti et al. 2022). Insulin's biological activity is initiated by binding to its receptor within the cell membrane. This binding triggers a cascade of intracellular signaling reactions, including activating glucose transporter enzymes and transcription factors such as PPARɣ. Any minor disruption in this signaling pathway can lead to a decrease in insulin sensitivity, subsequently leading to the aberrant increase in blood glucose level evident in T2D (Nailufar et al. 2011).

The enrichment analysis also showed enriched BP terms associated with inflammation, such as regulation of inflammatory response (GO:0050727), negative regulation of inflammatory response (GO:0050728), and inflammatory response (GO:0006954). Inflammation emerges as a common thread between obesity and insulin resistance (IR) since the increase in adipose tissue mass may induce chronic activation of the innate immune system, ultimately leading to IR and T2D. An increase in plasma levels of various cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP), indicates low-grade inflammation, which is a common characteristic of T2D. TNF-α is an inflammatory cytokine linked to obesity and IR. The liver primarily generates CRP, and its elevated levels have been correlated with several diseases, including obesity and diabetes (Calle and Fernandez 2012).

BP terms related to glucose metabolism include positive regulation of glucose metabolic process (GO:0010907), positive regulation of glucose import (GO:0046326), regulation of glucose metabolic process (GO:0010906), positive regulation of glucose transmembrane transport (GO:0010828), and regulation of glucose import (GO:0046324). In contrast, several BP terms are representative of lipid metabolism. These include response to lipid (GO:0033993), cellular response to lipid (GO:0071396), regulation of lipid storage

(GO:0010883), positive regulation of lipid metabolic process (GO:0045834), and regulation of lipid metabolic process (GO:0019216). The pancreatic endocrine cell hormones, glucagon, and insulin play crucial roles in regulating glucose and lipid metabolism. Insulin, in particular, acts as a critical regulator by inhibiting glucagon secretion and promoting the storage of lipids and carbohydrates. Conversely, glucagon facilitates gluconeogenesis, contributing to glucose homeostasis. This interplay between insulin and glucagon ensures the proper regulation of glucose and lipid metabolism, maintaining overall metabolic health (Parhofer 2015).

Finally, the molecular docking analysis demonstrated that quercetin, myricetin, chrysin, apigenin, and daidzein exhibited good to satisfactory binding strength with AKT1, EGFR, PTGS2, and PPARG, as evidenced by a Vina score of < −5.00 kcal/mol. The Vina score indicates the binding strength, which is the basis for categorizing binding strength as follows: potential (affinity of < −4.25 kcal/mol), good (affinity of < −5.00 kcal/mol), and satisfactory (affinity of < −7.00 kcal/mol) (Liu et al. 2021). The Vina score reflects the cumulative contribution of several interaction types, including hydrophobic contacts, van der Waals forces, and hydrogen bonds, which together determine the predicted binding affinity of the ligand–protein complex. Although the CB-Dock output does not explicitly display the interatomic interactions, the favorable scores obtained in this study (−6.5 to −8.1 kcal/mol) suggest that these forces collectively contribute to stable binding (Trott and Olson 2010). The molecular docking results validated the possibility that the crucial compounds can mediate the amelioration of T2D by targeting several T2D-related genes.

4. Conclusions

Our findings highlighted several key species, such as *Bacteroides uniformis* (quercetin producer), *Bifidobacterium adolescentis* and *Bifidobacterium breve* (daidzein producers), *L. plantarum* (10-oxo-11-octadecenoic acid producer), and *L. paracasei* (10-keto-12Z-octadecenoic acid producer) that can target crucial proteins such as AKT1, EGFR, PTGS2, and PPARG. Enrichment analysis revealed that these proteins participate in major biological routes, including the PI3K-Akt cascade, insulin signaling, and pathways governing inflammatory responses and glucose regulation. Overall, the results suggest that metabolites generated by specific gut microbes may influence host metabolic pathways and represent valuable candidates for future therapeutic development in type-2 diabetes. However, we used online databases that are continuously updated. Since the submission of this manuscript, these databases may have undergone several updates. Consequently, the findings presented here may not fully reflect the most current information available. Future research could revisit the analysis using updated datasets to validate and refine the findings.

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Authors' contributions

NW searched the databases and references, collected and analyzed the data, developed the method, created the illustrations, and drafted the manuscript. ST and STS provided technical support. RRT suggested the research topic and was responsible for supervising. All authors reviewed the manuscript and offered insightful revision recommendations.

Competing interests

The authors declare no competing interests.

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