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An Integrated Approach of Network Pharmacology and Molecular Docking Analyses for Identification of *Lepidium sativum* L. Antidiabetic Molecular Targets

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Abstract

Lepidium sativum L. is one of the most important medicinal plants with many reported pharmacological activities. One of these activities is the antidiabetic activity which is not extensively studied. Therefore, this study aims at identifying the mechanism underlying *L. sativum* antidiabetic activity using network-based pharmacology and molecular docking. Network pharmacology analysis revealed that the hit antidiabetic constituents in *L. sativum* were quercetin, resveratrol, apigenin, luteolin, and linoleic acid. Whereas PPARA, PIK3CA, PIK3CB, AKT1, and GSK3B were the most enriched diabetes-related genes. In addition, the most significant diabetes-related pathways were metabolic pathways and insulin resistance. Molecular docking of hit *L. sativum* constituents on the most enriched diabetes-related genes' active sites demonstrated that the most stable interactions were possessed by quercetin. This study provides illustration of *L. sativum* molecular mechanisms for alleviation of diabetes and its complications for the first time through network pharmacology analysis accompanied with molecular docking and lays the foundation for future *in vivo* and clinical research.

Keywords: *Lepidium sativum* L., Diabetes, Network pharmacology, Molecular docking, KEGG pathways.

1. Introduction

Diabetes mellitus is a chronic disease that is identified as disturbances in carbohydrates, proteins, and fats metabolism (Kooti et al., 2016). It is characterized by a rise in blood glucose level that can occur postprandially or during fasting and caused by insufficient or ineffective insulin (Kooti et al., 2016). This long-term hyperglycemic state leads to failure and damage in many organs such as kidney, heart, nerves, retina, and blood vessels (Alam et al., 2014). Diabetes can now be managed with a variety of therapies, including insulin therapy, medications, and nutrition therapy (Kooti et al., 2016). Some of these therapies exhibit many drawbacks, such as toxicity, side effects, and medication resistance (Moradi et al., 2018). Therefore, alternative diabetes treatment methods are required. Nowadays, the use of medicinal plants as preventive and therapeutic agents for diabetes is remarkably increasing. This is because medicinal plants showed effectiveness in the management of diabetes and its complications, they are also inexpensive and almost without adverse effects (Tabatabaei-Malazy et al., 2016).

'Garden cress', or *Lepidium sativum* L., is a member of Brassicaceae family (Vazifeh et al., 2022). It is one of the most widely used medicinal plants in the Arab countries where it is commonly known as "Hab al Rashad" (Abdallah et al., 2020).

Egyptians have ingested *L. sativum* since ancient times for a variety of medicinal and nutritional characteristics (Painuli et al., 2022). It is traditionally used in folk medicine to alleviate stomachaches, asthma, diabetes, pneumonia, bronchitis, pain, dysentery, uterus tumors, cough, ulcers, wounds, hemorrhoidal hemorrhage, dysmenorrhea, sciatica, dermatomycosis, and nasal polyps (Painuli et al., 2022; Vazifeh et al., 2022). In addition, the seeds and leaves are claimed to have aphrodisiac, laxative, galactagogue and diuretic activities (Mali et al., 2007). They are also suggested for mitigation of headache, malaria, sore throat, syphilis, chest complaints, rheumatism, inflammation, and muscular pains (Mali et al., 2007). Pharmacological studies revealed that *L. sativum* possesses anti-diarrheal, hepatoprotective, cardiotonic, antioxidant, hypotensive, antimicrobial, bronchodilator, and hypoglycemic activities (Mali et al., 2007; Painuli et al., 2022; Vazifeh et al., 2022). These activities are attributed to various *L. sativum* phytoconstituents, such as alkaloids, saponins, anthracene glycosides, proteins, flavonoids, glucosinolates, carbohydrates, saturated and essential fatty acids (Abdallah et al., 2020; Painuli et al., 2022).

Many *in vitro* and *in vivo* studies have investigated the antidiabetic effect of *L. sativum*. For example, Mishra et al. demonstrated that *L. sativum* seeds extract was able to lessen the complications associated with diabetes by controlling the balance of plasma redox. It was found to regulate blood glucose, plasma alkaline phosphatase levels, and plasma creatinine, thus improving the renal function when compared to diabetic control rats (Mishra et al., 2017) In addition, Eddouks et al. presented that *L. sativum* seeds' aqueous extract exhibited a strong hypoglycemic impact in both healthy and streptozotocin-induced diabetic rats without changing basal plasma insulin concentrations (Eddouks et al., 2005). Moreover, the assessment of *L. sativum* seeds' ability to slacken the starch hydrolysis to glucose in diabetic patients was done by *in vitro* studying their effect on the rate of starch hydrolysis. *L. sativum* seeds were discovered to significantly lower the hydrolysis of starch by 41% (Mali et al., 2007). Furthermore, in a rat model of alloxan-induced diabetes, Attia et al. showed that the methanolic extract of *L. sativum* seeds decreased blood sugar and corrected all biochemical and histological diabetes-induced complications (Attia et al., 2019). Another research carried out on albino male rats with hypercholesterolemia demonstrated the *L. sativum* seed extract's ability to reduce blood glucose levels and improve lipid profiles (decrease in TGs, cholesterol, LDL, and raise in high density lipoprotein cholesterol (HDL)) when compared to the control group (Amawi, 2012).

The complex nature of plant extracts makes it challenging to understand their role in the mitigation of various diseases. This might be because of the plant extracts' ability to simultaneously affect many targets or the phytoconstituents' synergism (Caesar et al., 2019). Recently, network pharmacology-based analysis has been successfully employed to anticipate the plant metabolome's disease pathways and molecular targets by allowing the compound-target gene-disease network to be imagined (Hopkins, 2008; Li et al., 2014). Network pharmacological analysis has proven helpful in explaining how various medicinal plants aid to palliate a variety of ailments (Chandran and Patwardhan, 2017; El-Banna et al., 2022; Ibrahim and El-Banna, 2021; Shawky et al., 2020).

The purpose of this study is to allow for illustration of the mechanism underlying *L. sativum* effect in alleviation of diabetes and its complications using an integrated strategy of network pharmacology and molecular docking analyses for the first time.

2. Methods

2.1. Assemblage of an in-house database for *L. sativum*

Based on a prior literature analysis on the metabolome of *L. sativum* (Abdallah et al., 2020; Ait-Yahia et al., 2018; Kadam et al., 2018; Painuli et al., 2022; Rajasekaran and Suresh, 2022), a database of 113 components was created (Table S1). By using PubChem (https://pubchem.ncbi.nlm.nih.gov/) and ChEMBL (https://www.ebi.ac.uk/chembl/), the 2D structures of these components were verified. Afterwards, Schrodinger software (2017A) was applied to convert these structures to the SMILES format.

2.2. ADME and drug-likeness filtration

Qikprop software (Schrodinger suite 2017A) was employed to filter *L. sativum* database phytoconstituents by following Lipinski's rule of five (Lipinski, 2004) and calculating the criteria of absorption, distribution, metabolism, and excretion (ADME). Herein, constituents that met no more than three of Lipinski's rule of five requirements were disqualified. Additionally, components with an anticipated oral bioavailability (OB) of no more than 30 were also precluded.

2.3. Network pharmacology-based analysis

2.3.1. Determination of diabetes-related genes for L. sativum filtered metabolites

STITCH database with the '*Homo sapiens*' species setting (http://stitch.embl.de/, ver. 5.0) (Shi et al., 2019) was hired to determine the target genes in relation with the filtered metabolites. Names, organism, IDs, and functions of the determined genes were got from UniProt (http://www.uniprot.org/) (Chandran and Patwardhan, 2017; Shi et al., 2019). The diabetes-related '*Homo sapiens*' proteins were the only ones that were kept. In addition, only '*Homo sapiens*' proteins associated with diabetes were used in GeneCards searches: The human gene database (http://www.genecards.org/) (Shi et al., 2019) to find more details on diabetes target genes. Then, the STRING database (https://string-db.org) was utilized to set up the network that presented the protein-protein interactions (PPI network) (Shawky et al., 2020).

2.3.2. Set up of networks and analysis of pathways

For examination of the multi-level mechanisms of action of *L. sativum* metabolites in diabetes mitigation, three different kinds of networks (constituent-gene, gene-pathway, and constituent-gene-pathway networks) were built *via* Cytoscape 3.8.2 (http://www.cytoscape.org/) (Ibrahim and El-Banna, 2021). The nodes in these networks stand for constituents, genes, and pathways, whilst the edges denote the interactions that exist among them. The parameters of the networks were calculated by Cytoscape's network analyzer plug-in. Cytoscape combined score of interactions is the parameter that denotes the significance of the built networks' nodes.

2.3.3. Gene ontology (GO) enrichment analysis for the determined target proteins

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (http://www.genome.jp/kegg/pathway.html) and the database for Annotation, Visualization and Integrated Discovery (DAVID) ver. 6.8 (https://david.ncifcrf.gov/) were searched for learning more about gene ontology and for finding the canonical pathways, cellular components, biological processes, and molecular functions that were closely linked to the target proteins (Chandran and Patwardhan, 2017; El-Banna et al., 2022; Ibrahim and El-Banna, 2021; Shawky et al., 2020; Shi et al., 2019). Pathways having *P*-values ≤ 0.01 were the only ones that were selected.

2.4. Molecular docking studies

The crystal structures of the most enriched diabetes-related targets identified from network-based pharmacology were gained from Protein Data Bank (PDB).

These identified targets were peroxisome proliferator-activated receptor alpha (PPARA, PDB ID: 6L36), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA, PDB ID: 6OAC), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PIK3CB, PDB ID: 4BFR), RAC-alpha serine/threonine-protein kinase (AKT1, PDB ID: 3O96), and glycogen synthase kinase 3 beta (GSK3B , PDB ID: 6GJO).

Each protein's crystal structure was chosen based on the highest available resolution. Protein preparation module of Schrodinger's Maestro molecular modeling suit (Schrödinger) was hired for preparation of the most enriched proteins' crystallographic structures. At first, protein preprocessing involved bond orders and hydrogens assigning, as well as making zero order bonds to metals and disulfide bonds. The active site was cleared of any water molecules away from 5 Å.

PROPKA was used to assign hydrogen bonds at PH = 7. OPLS 3 force field was applied to minimize the energy until the relative mean standard deviation (RMSD) of the minimized structure in comparison with the crystal structure was greater than 0.30 Å (Dawood et al., 2018). The receptor grid generating module was utilized to allocate the docking experiments' binding site, and the boxes encompassing the co-crystallized ligands' centroids were determined as the grids. To get the compounds' low energy structures, the 3D structures of the compounds were loaded as an SDF file into the LigPrep module of the Maestro molecular modeling program. Ionization states were regulated to create every conceivable state at pH 7. The extra-precision (XP-Glide) module of Glide docking program enclosed in the Maestro molecular modeling package was hired to simulate the molecular docking. The Maestro interface was applied to view the ligand-target interactions, involving hydrophobic interactions, ion pair interactions, hydrogen bonds, and the ligands' binding modes.

3. Results and discussion

Construction of a database containing 113 compounds was accomplished by prior literature analysis on the metabolome of *L. sativum*.

3.1. ADME filtration of *L. sativum* constituents

The ADME attributes of *L. sativum* constituents were evaluated through the use of the QikProp module, that gauges several physiochemical characteristics which indicate a compound's drug-likeness. Lipinski's rule of five provides a summary of these physiochemical characteristics. Based on Lipinski's rule of five, a compound should possess fewer than five hydrogen-bond donors (Hdon), fewer than ten hydrogen-bond acceptors (Hacc), a molecular weight fewer than 500 Da, 10 or lower rotatable bonds (RBN), and a computed log P (ClogP) fewer than five in order to be active (Lipinski, 2004). Only compounds that complied with three or more of the aforementioned characteristics were maintained in the database. *L. sativum* constituents' oral bioavailability (OB) was also estimated (Yang et al., 2021). It shows how much of a drug dosage taken orally reaches the therapeutic site of action without change. The compounds that retained in the database are the ones that have OB ≥ 30%. Out of the 113 compounds in the database, only 98 compounds met the above criteria (Table S1). Therefore these 98 compounds were forwarded to network-based pharmacological analysis.

3.2. Recognition of diabetes-related genes of *L. sativum* constituents through network pharmacology

Through the use of the search findings gained from the STITCH 5.0 database (Kuhn et al., 2008), a compound-target network was built in an attempt to identify the diabetes-related target genes of *L. sativum* constituents. The function of each recognized target gene and its relationship to diabetes were determined using the UniProt and GeneCards databases (Bairoch et al., 2005). 'Combined score' is the parameter used in STITCH 5.0 database for the judgment of the strength of interactions among compounds and genes where more powerful interactions exhibit greater values of combined scores. Herein, the constituents exhibiting combined interaction scores ≥ 0.5 were the only ones that were kept (Table 1). The compound-target gene (C-T) network consisted of 48 nodes (21 constituents and 27 genes) and 245 edges (Figure 1) with an average of 2.417 targets for each constituent illustrating the multi-target attribute of the constituents under study.

The compound-target (C-T) network demonstrated that quercetin (flavonoid) had the largest percentage of C-T interactions (32%), then resveratrol (21%) (stilbenoid), apigenin (5%) (flavonoid), luteolin (5%) (flavonoid), and linoleic acid (4%) (polyunsaturated fatty acid) (Figure 2A). For verification of these results generated from network pharmacology analysis, papers studying the relation of these hit constituents to diabetes and its complications were searched for in PubMed (Table 2). For example, quercetin was found to reduce the risk of type 2 diabetes mellitus by preventing pancreatic iron buildup and ferroptosis in pancreatic cells (Li et al., 2020). It also reduced the testicular abnormality that diabetes causes in Wistar rats through the mitochondrial-mediated apoptotic pathway (Ojo and Olorunsogo, 2021). In addition, resveratrol boosted insulin sensitivity while lowering fasting glucose, insulin, and insulin resistance when compared to a placebo (Hoseini et al., 2019). It also significantly reduced oxidative stress, chronic inflammation, and the expression of associated microRNAs in diabetic patients. Therefore, administration of resveratrol with oral hypoglycemic drugs may help to lessen the complications of diabetes (Mahjabeen et al., 2022). Moreover, apigenin mended streptozotocin-induced diabetic nephropathy in rats through TGF-β1-MAPK-fibronectin and MAPK-NF-κB-TNF-α pathways (Malik et al., 2017). It also showed the ability to reduce the cognitive impairment caused by diabetes in rats by repressing the oxidative stress, nitric oxide, and apoptotic cascades synthase pathway (Mao et al., 2015). Furthermore, luteolin provided protection from diabetic cardiomyopathy through preventing NF-κB -mediated inflammation and triggering Nrf2-mediated antioxidant responses (Li et al., 2019) It also aided in wound healing by reducing oxidative stress and inflammation via NF-κB inactivation and Nrf2 upregulation (Chen et al., 2021) Additionally, linoleic acid was able to affect insulin sensitivity as well as lipid and glucose metabolism in patients with type-2 diabetes mellitus (Belury et al., 2003). It also reduced the injury of podocytes brought on by hyperglycemia by inhibiting the NLRP3 inflammasome pathway (Yu et al., 2019).

Network pharmacology analysis also revealed that the most enriched diabetes-related genes showing the largest C-T interactions percentages were PPARA (22%), PIK3CA (10%), PIK3CB (10%), AKT1 (10%), and GSK3B (9%) (Figure 2B). These are familiar diabetes-related targets where PPARA is a member of transcription factors which are important for lipid and glucose metabolism.

Table 1. Target proteins for *L. sativum* phytoconstituents

Short name of protein	Full name of protein	Interacting compound (s) (combined interaction score)
AKR1B1	Aldo-keto reductase family 1 member B1	Quercetin (1), luteolin (1), (E)-p-coumaric acid (1)
AKT1	RAC-alpha serine/threonine-protein kinase	Quercetin (1),
ALPL	Alkaline phosphatase, tissue-nonspecific isozyme	(-)-Catechin (1)
BRAF	Serine/threonine-protein kinase B-raf	Quercetin (1)
CD38	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1	Luteolin (1)
CDK6	Cyclin-dependent kinase 6	Apigenin (1)
CFTR	Cystic fibrosis transmembrane conductance regulator	Apigenin (1)
CNR1	Cannabinoid receptor 1	Linoleic acid (0.65)
FOS	Proto-oncogene c-Fos	Ferulic acid (0.74)
FUT4	Alpha-(1,3)-fucosyltransferase 4	4`-O-Methyl-epigallocatechin (0.5)
FUT7	Alpha-(1,3)-fucosyltransferase 7	4`-O-Methyl-epigallocatechin (0.5)
GLO1	Lactoylglutathione lyase	Caffeic acid (0.53), quercetin (1), ferulic acid (0.74), apigenin (0.78), luteolin (1), kaempferol (0.78)
GSK3B	Glycogen synthase kinase-3 beta	Quercetin (1), apigenin (1), luteolin (1)
IGF1R	Insulin-like growth factor 1 receptor	Quercetin (1)
NOX4	NADPH oxidase 4	Quercetin (1), apigenin (1), luteolin (1), kaempferol (0.78)
PARP1	Poly [ADP-ribose] polymerase 1	Luteolin (1)
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	Resveratrol (1)
PIK3CB	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform	Resveratrol (1)
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit alpha	Quercetin (1)
PLA2G1B	Phospholipase A2	Quercetin (1)
PPARA	Peroxisome proliferator-activated receptor alpha	Linoleic acid (1), oleic acid (1), eicosenoic acid (1), caprylic acid (1), nonanoic acid (1), myristic acid (1), pentadecanoic acid (1), palmitoleic acid (1), palmitic acid (1)
PPARD	Peroxisome proliferator-activated receptor delta	Linoleic acid (1), oleic acid (1), eicosenoic acid (1), palmitoleic acid (1)
PPARG	Peroxisome proliferator-activated receptor gamma	Linoleic acid (1), oleic acid (1), eicosenoic acid (1), palmitoleic acid (1)
PTPN1	Tyrosine-protein phosphatase non-receptor type 1	Caffeic acid (1)
RPS6KA3	Ribosomal protein S6 kinase alpha-3	Kaempferol 7-O-rhamnoside (0.56), kaempferol (0.51)
SLC5A1	Sodium/glucose cotransporter 1	Semilepidinoside A (0.51)
TLR2	Toll-like receptor 2	Linoleic acid (0.65), caprylic acid (1), nonanoic acid (1), myristic acid (1), pentadecanoic acid (1), palmitic acid (1)

Table 2. Summary of PubMed review on top hit *L. sativum* phytoconstituents in diabetes remediation

Compound	Model	Reference	Mechanism
Quercetin	Type 2 diabetes (T2DM) mice model	PMID: 32992479	Quercetin reduced the risk of type 2 diabetes mellitus by preventing pancreatic iron buildup and ferroptosis in pancreatic cells.
	Wistar rats with streptozotocin (STZ)-induced diabetes	PMID: 34275146	Quercetin reduced the testicular abnormality that diabetes causes in Wistar rats through the mitochondrial-mediated apoptotic pathway.
	High-fat-high-fructose diet (HFFD)-fed C57BL/6 mice and palmitic acid (PA)-treated HepG2 cells.	PMID: 35124182	Quercetin and EGCG administered together could reduce insulin resistance through IRS-1/Akt/FOXO1 pathway.

	Placentas from female rats with streptozotocin (STZ)-induced gestational diabetes mellitus (GDM)	PMID: 33640737	Following quercetin treatment, the placenta of rats with gestational diabetes mellitus showed better histological structure and an upregulation of adiponectin and adiponectin receptors
	α-amylase (in vitro) and Wistar rats with streptozotocin (STZ)-induced diabetes (in vivo)	PMID: 32795923	-In an in vitro study, swertiamarin and quercetin (CSQ) administered together were discovered to have a significant percentage of inhibition to α-amylase. - A marked reduction in the blood glucose levels was observed in the CSQ treated mice. - Decline in the levels of triglycerides, low-density lipoprotein (LDL), total cholesterol and a rise in high-density lipoprotein (HDL) cholesterol level was observed in CSQ treated mice. - CSQ treated groups were able to boost antioxidant defense by raising the levels of blood SOD, GSH, GPx and catalase and lowering the levels of lipid peroxide.
Resveratrol	Patients having type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD)	PMID: 31486447	-Resveratrol boosted insulin sensitivity while lowering fasting glucose, insulin, and insulin resistance when compared to a placebo. -PPAR- and sirtuin 1 (SIRT1) were upregulated by resveratrol in the peripheral blood mononuclear cells (PBMCs) of diabetic patients suffering from coronary heart disease.
	Blood obtained from 8 healthy volunteers and 10 patients with type 2 diabetes	PMID: 35458194	Patients with type 2 diabetes may experience decreased platelet function and thrombus formation due to resveratrol's suppression of platelet metabolism and TXA2 release.
	A randomized, double blinded placebo-controlled parallel group trial.	PMID: 35240291	Resveratrol helped to enhance glycemic control through insulin resistance reduction. It could also significantly reduce oxidative stress, chronic inflammation, and the expression of associated microRNAs in diabetic patients. Therefore, administration of resveratrol with oral hypoglycemic drugs may help to lessen the complications of diabetes.
	Diabetic mice	PMID: 31844893	Resveratrol reduced cell centrosome amplification associated with diabetes through PKCα-p38 to c-myc/c-jun pathway inhibition.
	Type 2 diabetic men (19) on oral glucose-lowering medication	PMID: 21385509	In those with type 2 diabetes, resveratrol raised insulin sensitivity, lowered oxidative stress, and activated the Akt pathway.
Apigenin	Streptozotocin (STZ)-induced diabetic rats.	PMID: 28566504	Apigenin mended streptozotocin-induced diabetic nephropathy in rats through TGF-β1-MAPK-fibronectin and MAPK-NF-κB-TNF-α pathways.
	Zucker diabetic fatty rats	PMID: 32507768	In renal tubular cells of diabetic rats, apigenin suppressed CD38 and reduces mitochondrial oxidative stress via restoring the intracellular NAD+/NADH ratio and Sirt3 activity.
	Streptozotocin (STZ)-induced diabetic rats.	PMID: 26629041	Apigenin showed the ability to reduce the cognitive impairment caused by diabetes in rats by repressing the oxidative stress, nitric oxide, and apoptotic cascades synthase pathway.
	Rats with Streptozotocin (STZ)-induced diabetic cardiomyopathy (DCM)	PMID: 28176247	Apigenin reduced the fibrosis, overproduction of 4-hydroxynonenal, and cardiac dysfunction brought on by diabetes mellitus, along with the

			down-regulation of GPx, Bcl2, and SOD, the up-regulation of cleaved caspase3, MDA, and pro-apoptotic protein Bax, and contribution to the translocation of NF-κB.
Luteolin	C57BL/6 mice with Streptozotocin (STZ)-induced diabetes mellitus	PMID: 31009852	Luteolin provided protection from diabetic cardiomyopathy through preventing NF-κB -mediated inflammation and triggering Nrf2-mediated antioxidant responses.
	Streptozotocin-induced diabetic rats	PMID: 23415807	Long-term luteolin supplementation reduced oxidative stress and choline esterase (ChE) activity in diabetic mice, and that improved neuronal damage and cognitive performance.
	Streptozotocin-induced diabetic rats	PMID: 23296950	Luteolin reduced the effects of diabetes on the aorta, including dyslipidemia, elevated diastolic blood pressure, decreased response to Ach, and reduced NO production.
	Streptozotocin-induced diabetic rats	PMID: 34209369	Luteolin aided in wound healing by reducing oxidative stress and inflammation via NF-κB inactivation and Nrf2 upregulation.
	High glucose-induced MPC-5 cells	PMID: 30935950	Luteolin reduced the injury of podocytes brought on by hyperglycemia by inhibiting the NLRP3 inflammasome pathway.
Linoleic acid	Subjects with type 2 diabetes mellitus	PMID: 12514304	Linoleic acid was able to affect insulin sensitivity as well as lipid and glucose metabolism.
	N=17 Men & women (Non-obese, N=6; Obese, N=5; T2DM, N=6)	PMID: 11914742	Linoleic acid was able to enhance insulin sensitivity and reduce visceral adipose.
	N=55 Obese, postmenopausal women with T2DM Oral hypoglycemic medications	PMID: 19535429	Treatment with linoleic acid reduced insulin resistance and trunk adipose tissue while boosting lean mass and adiponectin.

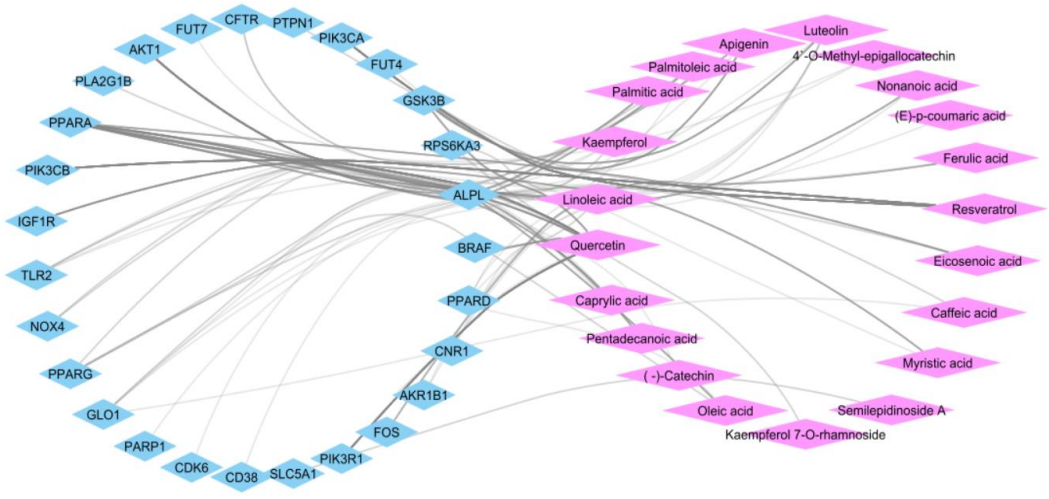


Figure 1: Network of compound–target gene interactions for *L. sativum* constituents by linking 21 compounds (purple colour) and 27 target proteins (blue colour

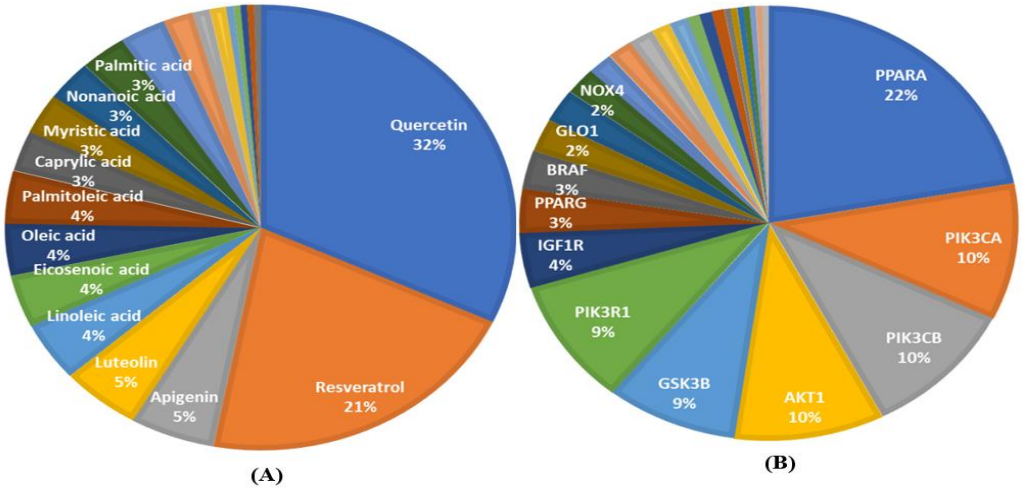


Figure 2: The distributions % of the compound–target gene (C–T) interactions on: (A) *L. sativum* constituents. (B) on the identified diabetes-related proteins.

They are expressed in a variety of cell kinds such as immune cells and pancreatic beta cells, where they control the differentiation of T cells and the release of insulin, respectively (Holm et al., 2020). In addition, phosphoinositide 3-kinases (PI3Ks) have grown to be the focus of numerous pharmaceutical therapies. Since PI3Ks are crucial for controlling glucose levels, this raises the possibility that they may also be involved in the development of diabetes mellitus (Maffei et al., 2018). PIK3CA is involved in signaling via insulin-receptor substrate (IRS) proteins (Maheshwari et al., 2017). Also, PIK3CB possesses a function in insulin signaling where it acts as scaffolding protein which does not require the activity of lipid kinase (Czupalla et al., 2003). Moreover, AKT is from the main factors governing lipid and glucose metabolism and it is the main focus of contemporary research studying diabetes and metabolic illnesses. It is primarily found in major organs involved in metabolism and is triggered by a variety of stimuli, such as cell movement, cell stress, and numerous medications and hormones which alter the metabolism of the cells (Miao et al., 2022). Furthermore, GSK3 was recognized by scientists as an essential therapeutic target for the treatment of diabetes. Insulin's inhibition of GSK3 promotes glycogen synthase's dephosphorylation, which activates it to turn UDP glucose into glycogen. Therefore, insulin resistance and lack of insulin are considered to be the reasons for type 2 diabetes (Maqbool and Hoda, 2017). The constructed protein-protein network revealed strong interactions among the recognized target genes (Figure 3). The signaling pathways and roles of the determined target genes were specified by carrying out KEGG pathways analysis (Kanehisa and Goto, 2000) which revealed the involvement of the target genes in 29 diabetes-related pathways (Table 3, Figure 4).

Metabolic pathways and insulin resistance were the most enriched pathways with the highest gene count, followed by mTOR signaling pathway and cAMP signaling pathway. The aforementioned networks were combined to create the compound–target–pathway network (Figure 5) which indicated that *L. sativum* phytoconstituents and diabetes-associated proteins and pathways were strongly correlated.

3.3. Gene ontology (GO) enrichment analysis for targets

Gene ontology enrichment analysis for the recognized 27 targets was conducted via DAVID bioinformatics resources with annotations restricted to *Homo sapiens* (Dennis et al., 2003). That enables the determination of the most enriched pathways and GO terms (biological processes, cellular components, and molecular functions) that possess the largest gene counts and log *p*-values. The GO enrichment analysis revealed 28 KEGG pathways, all with p-values ≤ 0.01, including insulin resistance, EGFR tyrosine kinase inhibitor resistance, and non-alcoholic fatty liver disease (Figure 6A). From figure 6B it could be observed that the most significant biological processes were signal transduction, positive regulation of gene expression, and insulin-like growth factor receptor signaling pathway. Whereas the most important cellular components were phosphatidylinositol 3-kinase complex, class IA, phosphatidylinositol 3-kinase complex, and plasma membrane. Whilst the most enriched molecular functions were insulin receptor substrate binding, RNA polymerase II sequence-specific DNA binding transcription factor binding, and transcription factor binding.

Table 3. KEGG pathway analysis of the recognized target genes

#Pathway ID	Pathway description	Observed gene count	False discovery rate	Matching proteins in network
hsa04931	Insulin resistance	8	2.47E-09	PIK3CA, PIK3CB, GSK3B, PTPN1, R PS6KA3, PPARA, PIK3R1, AKT1
hsa01521	EGFR tyrosine kinase inhibitor resistance	7	7.75E-09	PIK3CA, IGF1R, BRAF, PIK3CB, GSK3B, PIK3R1, AKT1
hsa04150	mTOR signaling pathway	8	1.28E-08	PIK3CA, IGF1R, BRAF, PIK3CB, GSK3B, RPS6KA3, PIK3R1, AKT1
hsa01522	Endocrine resistance	7	1.59E-08	PIK3CA,IGF1R,BRAF,PIK3CB,FOS,PIK3R1,AKT1
hsa04152	AMPK signaling pathway	7	6.28E-08	CFTR,PIK3CA,IGF1R,PPARG,PIK3CB,PIK3R1,AKT1
hsa04024	cAMP signaling pathway	8	8.27E-08	CFTR,PIK3CA,BRAF,PIK3CB,FOS,PPARA,PIK3R1,AKT1
hsa04910	Insulin signaling pathway	7	9.82E-08	PIK3CA,BRAF,PIK3CB,GSK3B,PTPN1,PIK3R1,AKT1
hsa04932	Non-alcoholic fatty liver disease	7	1.65E-07	PIK3CA,PIK3CB,FOS,GSK3B,PPARA,PIK3R1,AKT1
hsa04973	Carbohydrate digestion and absorption	5	3.31E-07	PIK3CA,SLC5A1,PIK3CB,PIK3R1,AKT1
hsa04015	Rap1 signaling pathway	7	9.34E-07	PIK3CA,IGF1R,BRAF,PIK3CB,CNR1,PIK3R1,AKT1
hsa04935	Growth hormone synthesis, secretion and action	6	9.34E-07	PIK3CA,PIK3CB,FOS,GSK3B,PIK3R1,AKT1
hsa04068	FoxO signaling pathway	6	1.30E-06	PIK3CA,IGF1R,BRAF,PIK3CB,PIK3R1,AKT1
hsa04210	Apoptosis	6	1.55E-06	PIK3CA,PIK3CB,FOS,PARP1,PIK3R1,AKT1
hsa04151	PI3K-Akt signaling pathway	8	1.95E-06	TLR2,PIK3CA,CDK6,IGF1R,PIK3CB,GSK3B,PIK3R1,AKT1
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5	8.95E-06	NOX4,PIK3CA,PIK3CB,PIK3R1,AKT1
hsa04066	HIF-1 signaling pathway	5	1.23E-05	PIK3CA,IGF1R,PIK3CB,PIK3R1,AKT1
hsa04923	Regulation of lipolysis in adipocytes	4	2.26E-05	PIK3CA,PIK3CB,PIK3R1,AKT1
hsa04014	Ras signaling pathway	6	2.31E-05	PIK3CA,IGF1R,PIK3CB,PLA2G1B,PIK3R1,AKT1
hsa04140	Autophagy	5	2.60E-05	PIK3CA,IGF1R,PIK3CB,PIK3R1,AKT1
hsa04071	Sphingolipid signaling pathway	4	0.00031	PIK3CA,PIK3CB,PIK3R1,AKT1
hsa04930	Type II diabetes mellitus	3	0.00042	PIK3CA,PIK3CB,PIK3R1
hsa04072	Phospholipase D signaling pathway	4	0.00071	PIK3CA,PIK3CB,PIK3R1,AKT1
hsa04630	JAK-STAT signaling pathway	4	0.00095	PIK3CA,PIK3CB,PIK3R1,AKT1
hsa03320	PPAR signaling pathway	3	0.0015	PPARG,PPARD,PPARA
hsa01100	Metabolic pathways	9	0.0041	CD38,PIK3CA,AKR1B1,PIK3CB,PLA2G1B,FUT7,FUT4,GLO1,ALPL
hsa04920	Adipocytokine signaling pathway	2	0.0238	PPARA,AKT1
hsa00562	Inositol phosphate metabolism	2	0.026	PIK3CA,PIK3CB
hsa04976	Bile secretion	2	0.0367	CFTR,SLC5A1
hsa04922	Glucagon signaling pathway	2	0.0456	PPARA,AKT1

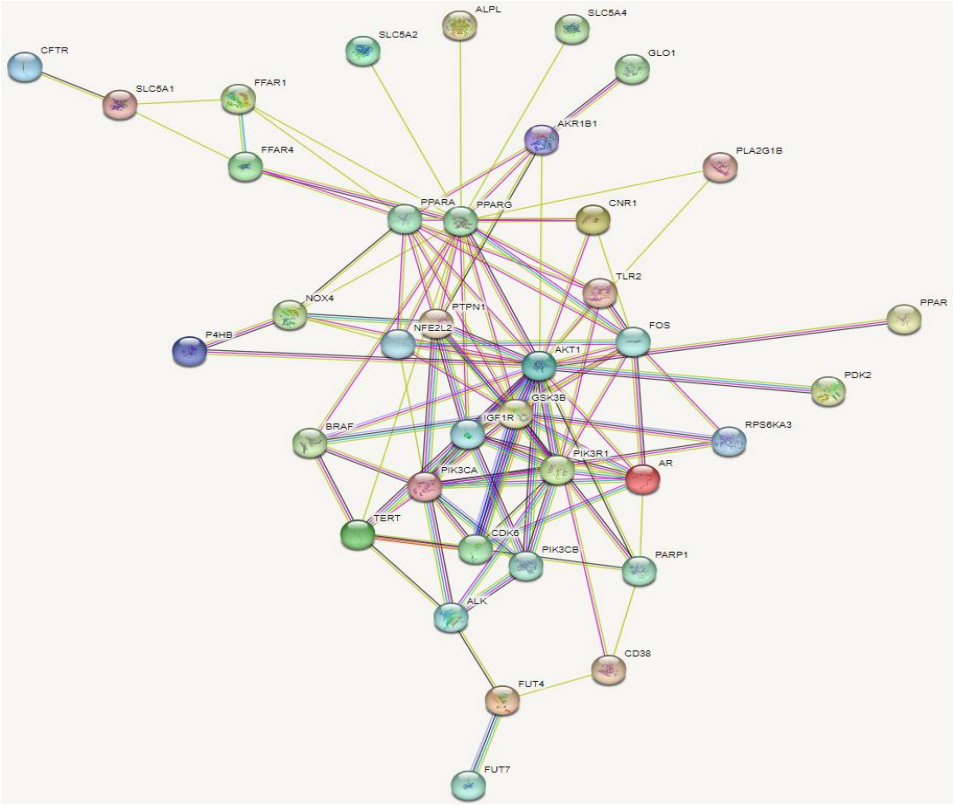


Figure 3: Protein–protein interaction (PPI) network of identified diabetes-related targets.

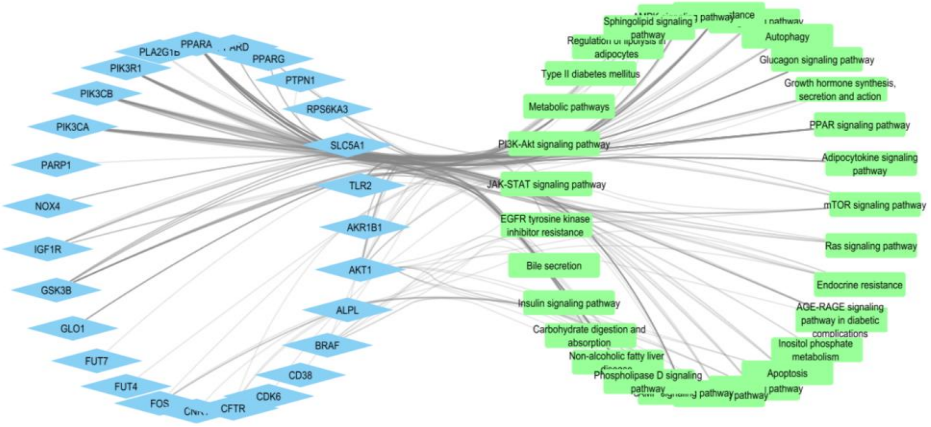


Figure 4: Gene–pathway network (genes are presented in blue color, pathways are presented in green color).

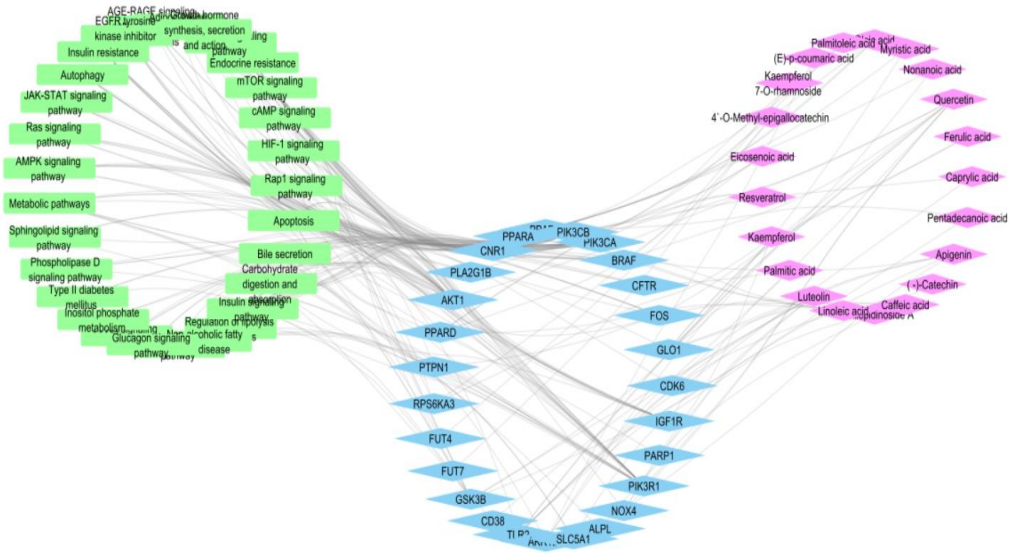
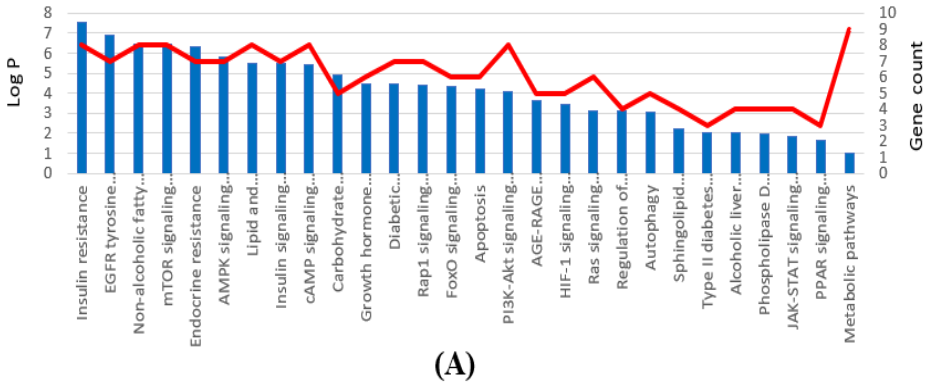
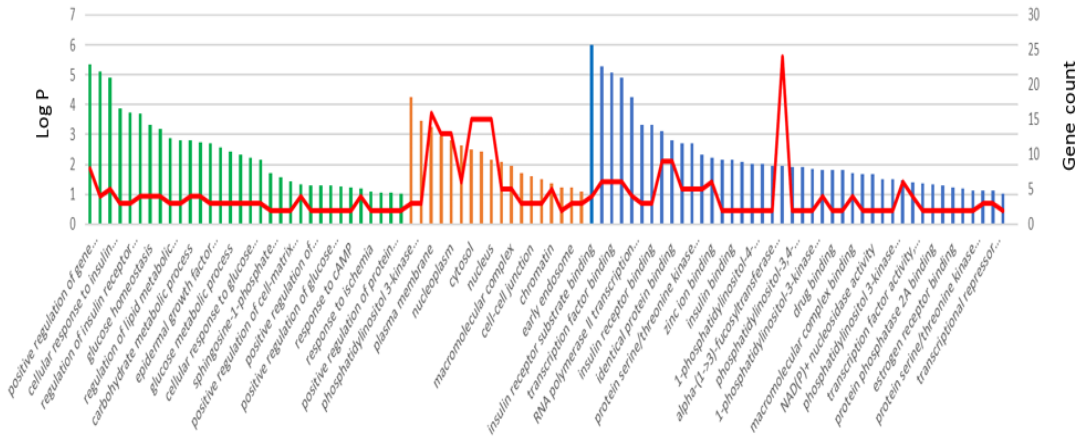


Figure 5: Compound–target–pathway network (compounds are represented in purple color, targets are presented in blue color and pathways are presented in green color).



(A)



(B)

Figure 6: (A) KEGG (blue) pathways analysis involved in diabetes. (B) GO enrichment analysis of identified diabetes-related targets. Biological processes are colored green, cellular components are orange and molecular functions are blue. The order of importance was ranked by -log 10 (*P*-value) with bar chart. The number of targets stick into each term with line chart

3.4. Molecular docking of hit *L. sativum* constituents on the most enriched diabetes-related genes' binding sites

The extra precision (XP) docking G scores of *L. sativum* hit constituents; quercetin, resveratrol, apigenin, luteolin, and linoleic acid, on the binding sites of most enriched diabetes-linked genes; PPARA, PIK3CA, PIK3CB, AKT1, and GSK3B, were calculated by means of the Glide module of the Schrodinger suite software. Table 4 illustrates that the lowest binding energy and the most stabilized interactions against these five genes were exhibited by quercetin. As can be shown from quercetin's 2D and 3D interaction diagrams against PPARA (peroxisome proliferator-activated receptor alpha) (PDB ID: 6L36) active site (Figure 7A), the stable interaction was due to two hydrogen bonds formation among 3`, 4`-OH groups and ASP453, and another hydrogen bond between 7-OH and Tyr314. There were also hydrophobic interactions with Ile447, Val444, Phe351, Ile354, Phe273, Tyr464, Leu460, Tyr314, Ala455, Leu456, and Ala454; polar interaction with Hie440, Ser280, and Gln277; and charged positive interaction with Lys448.

In addition, a stacking pi–pi interaction was found between the flavonoidal aromatic ring A and Hie440 (Wang et al., 2021).
Meanwhile, the stable interaction of quercetin in the active site of PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) (PDB ID: 6OAC) was due to hydrogen bonds formation between 4`-OH, 3-OH, 4-carbonyl group and Asp933, Glu849, Val851, respectively. Additional pair of hydrogen bonds was observed between 3`-OH and Asp933, Tyr836; and another pair was found between 5-OH and Val851. Hydrophobic interactions with Met922, Val850, Val851, Phe930, Ile848, Ile800, Ile932, Phe934, Tyr836, Trp780, and Met772; polar interaction with Ser854; charged negative interactions with Glu849, Asp933, and Asp810; charged positive interaction with Lys802; and a pi-pi stacking interaction between the aromatic ring A of the flavonoid moiety and Trp780 were also observed (Figure 7B) (Yang et al., 2020).

Moreover, the stabilization of quercetin in the PIK3CB (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta) (PDB ID: 4BFR) binding site was owing to a couple of hydrogen bonds between 3-OH and 4-carbonyl group and Lys799, and another couple among 4`-OH and Glu846, Val848. The stabilization also involved hydrophobic interactions with Met773, Ile845, Ile797, Ile930, Phe928, Val848, Trp781, Val847, Tyr833, and Met920; polar interaction with Thr853; charged positive interaction with Lys799; and charged negative interactions with Asp931, Glu846, and Asp917. A stacking pi–pi interaction among the flavonoidal aromatic ring B and Tyr833 was also observed (Figure 7C) (Yang et al., 2020).

In addition, quercetin occupied the binding site of AKT1 (RAC-alpha serine/threonine-protein kinase) (PDB ID: 3O96) with a pair of hydrogen bonds between 3`,4`-OH groups and Gln79, and another pair between 5,7-OH groups and Ser205, Thr211, respectively. The binding also involved hydrophobic interactions with Leu264, Tyr263, Trp80, Leu210, Ile290, Ala212, Tyr272, Val270, and Ile84; polar interactions with Thr81, Thr82, Gln79, Asn54, Thr211, and Ser205; charged positive interaction with Lys268; charged negative interaction with Asp292; and a pi-pi stacking interaction between flavonoidal aromatic ring A and Trp80 (Figure 8A) (Yang et al., 2020).

However, the binding of quercetin with GSK3B (glycogen synthase kinase 3 beta) (PDB ID: 6GJO) was through three hydrogen bonds between 3`,5, 7-OH groups and AsnA 186, AspA 133, and ValA 135, respectively; hydrophobic interactions with PheA 67, IleA 62, TyrA 134, ValA 135, LeuA 188, AlaA 83, ValA 110, LeuA 132, CysA 199, and ValA 70; polar interactions with AsnA 64, SerB 261, AsnA 186, and GlnA 185; charged negative interactions with AspA 133 and AspA 200; charged positive interactions with LysA 85 and LysA 183; in addition to a pi-pi stacking interaction between flavone aromatic ring B and PheA 67 (Figure 8B) (Johnson et al., 2011).

4. Conclusion

Network pharmacology and molecular docking analyses are considered as helpful, convenient, and low-cost tools for exploration of plants role and mode of action in diverse diseases. *L. sativum* is rich in valuable metabolites that can be exploited to alleviate diabetes and its complications. Additional in-depth *in vivo* and clinical investigations are necessary to assure the antidiabetic role of the recognized *L. sativum* hit constituents.

Conflict of interest

There is no conflict of interest.

Table 4. XP G scores of the top hit compounds in the compound–target network against the most enriched diabetes-associated target proteins

	Peroxisome proliferator-activated receptor alpha (6L36)	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (6OAC)	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (4BFR)	RAC-alpha serine/threonine-protein kinase (3O96)	Glycogen synthase kinase 3 beta (6GJO)
Quercetin	-11.433	-11.738	-10.790	-10.401	-9.863
Luteolin	-11.195	-10.429	-9.535	-10.070	-9.770
Apigenin	-9.404	-9.452	-8.987	-8.881	-9.156
Resveratrol	-8.155	-9.370	-7.431	-7.884	-6.563
Linoleic acid	-6.032	-4.544	-6.827	-5.082	-5.901

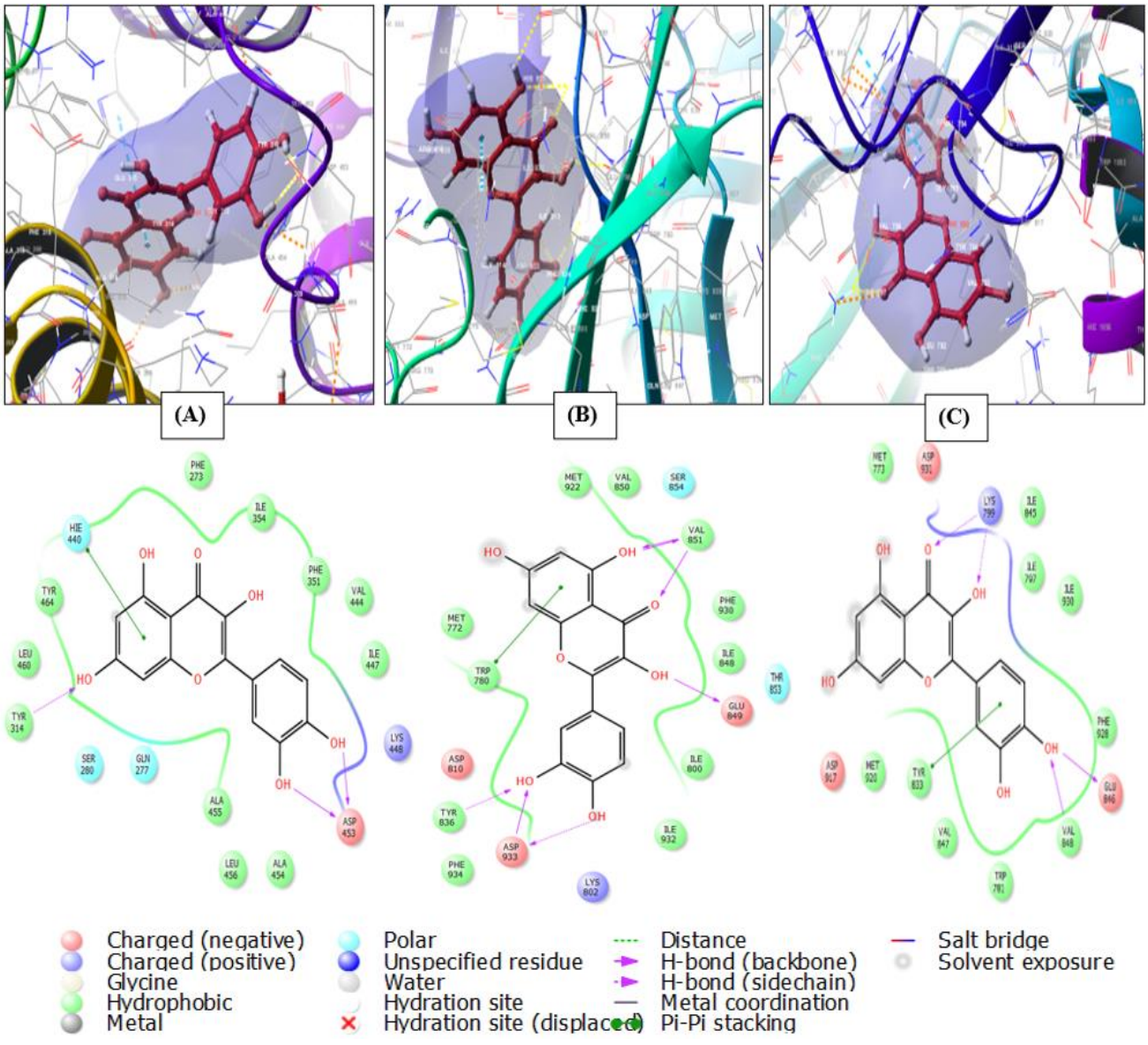


Figure 7: 2D and 3D interaction diagrams of quercetin in the active site of (A) peroxisome proliferator-activated receptor alpha (PBD ID 6L36) (B) phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PDB ID 6OAC) (C) phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PDB ID 4BFR).

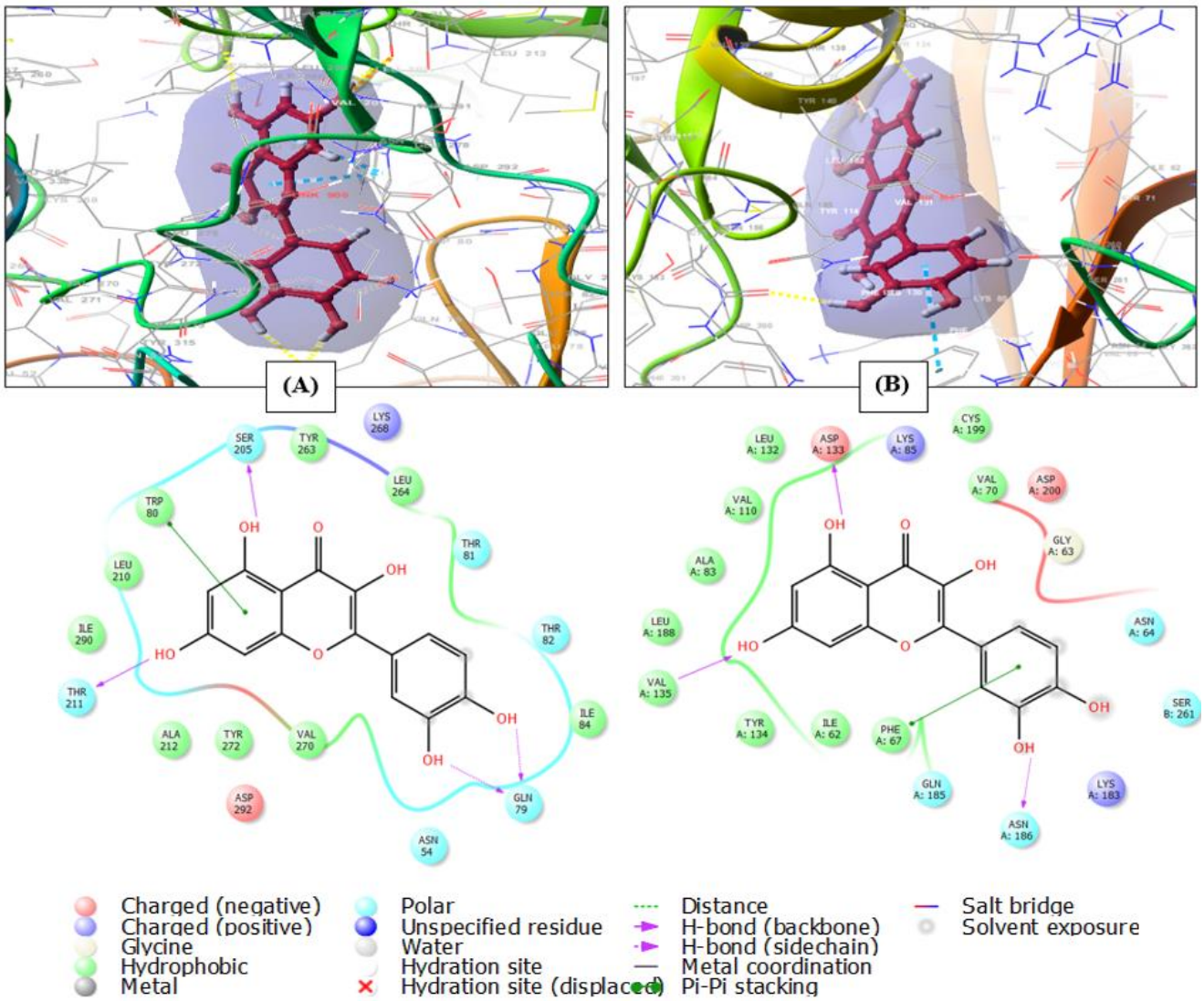


Figure 8: 2D and 3D interaction diagrams of quercetin in the active site of (A) RAC-alpha serine/threonine-protein kinase (PDB ID 3O96) (B) glycogen synthase kinase 3 beta (PDB ID 6GJO).

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