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In silico targeting of CYP19A1 and PTPN1 by Nigellidine: A network-based exploration of its antidiabetic pathway interactions

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Type 2 diabetes mellitus (T2DM) is a common metabolic health problem that is seen across the world with high levels of complications like cardiovascular disease, nephropathy and neuropathy. Nigellidine is an alkaloid of *Nigella sativa* and has shown good pharmacological potential. The integrative *in silico* strategy was used in this work and included the ADME/toxicity forecasting, network pharmacology, PPI analysis, and pathway enrichment. Also, molecular docking and molecular dynamics simulations were performed to determine important molecular targets and pathways that Nigellidine can influence to maintain T2DM. The structural and physicochemical properties of Nigellidine were obtained in PubChem and evaluated through Swiss ADME to achieve the drug-likeness. Swiss Target Prediction was used to predict potential molecular targets, and DisGeNET was used to get T2DM-related genes. Venn analysis was used to find similar targets. STRING was used to construct a protein-protein interaction (PPI) network that was analyzed in Cytoscape. hub genes were enriched by Gene Ontology (GO) and pathway analysis, KEGG, using DAVID. To measure binding affinities Molecular docking and dynamic simulation were carried out. Nigellidine exhibited good drug-like properties, having a molecular weight of 294.35 g/mol and TPSA of 47.16 Å². It was identified to have 15 common targets, each of which was a central node, such as CYP19A1, PTPN1 and HSD11B1. These were associated with insulin signalling, glucose metabolism, and inflammation using GO and KEGG analyses. Docking was very much affinity-wise to CYP19A1 (-9.45 kcal/mol), PTPN1 (-9.05 kcal/mol), and HSD11B1 (-8.84 kcal/mol). Nigellidine has been shown to be a dynamically favorable endlessly stabilized complex with CYP19A1. This *in silico* integrated study indicates that Nigellidine has multi-targeted antidiabetic effects, and it should be examined further preclinically and clinically to manage T2DM.

Keywords: Global Burden, Molecular docking, Molecular dynamic simulation, Natural product, *Nigella sativa*, PPI network

Diabetes mellitus (DM) has become one of the most burning issues of the 21st century among the global health problems. It is an acute metabolic disorder characterized by the constantly increased glucose concentration in the blood, which has reached the level of an epidemic. The International Diabetes Federation (IDF) estimates that, as of 2019, there were 463 million adults with diabetes, which is expected to rise to 700 million by 2045[1]. The effects of the disease extend beyond statistics, since there is a high possibility of major complications of the disease (cardiovascular diseases, kidney damage (nephropathy), eye damage (retinopathy), nerve damage (neuropathy)) that result in a lower quality of life and a greater risk of mortality[2]. The global healthcare burden of diabetes is not equally allocated among the nations, with the low and middle-income nations bearing a disproportionate load. Not only do these regions experience the increasing prevalence of diabetes in the area, but these areas are also forced to deal with chronic problems of infectious diseases and malnutrition[3]. The financial cost is enormous in economic terms, with the amount of money spent on healthcare annually to treat diabetes globally estimated at 760 billion annually and is expected to surpass 1 trillion by 20404. There are two broad categories of diabetes, namely Type 1 and Type 2. Insulin-dependent diabetes (Type 1 diabetes, T1D) is an autoimmune disease where the body's immunity attacks and kills the insulin-producing beta cells within the pancreas[5]. This leads to minimal or no insulin secretion, hence lifetime insulin treatment is mandatory. T2D is a diabetes variant that is a result of insulin resistance and relative lack of insulin production, wherein insulin resistance progressively worsens in response throughout the body over time and the pancreatic production of insulin by the 30per

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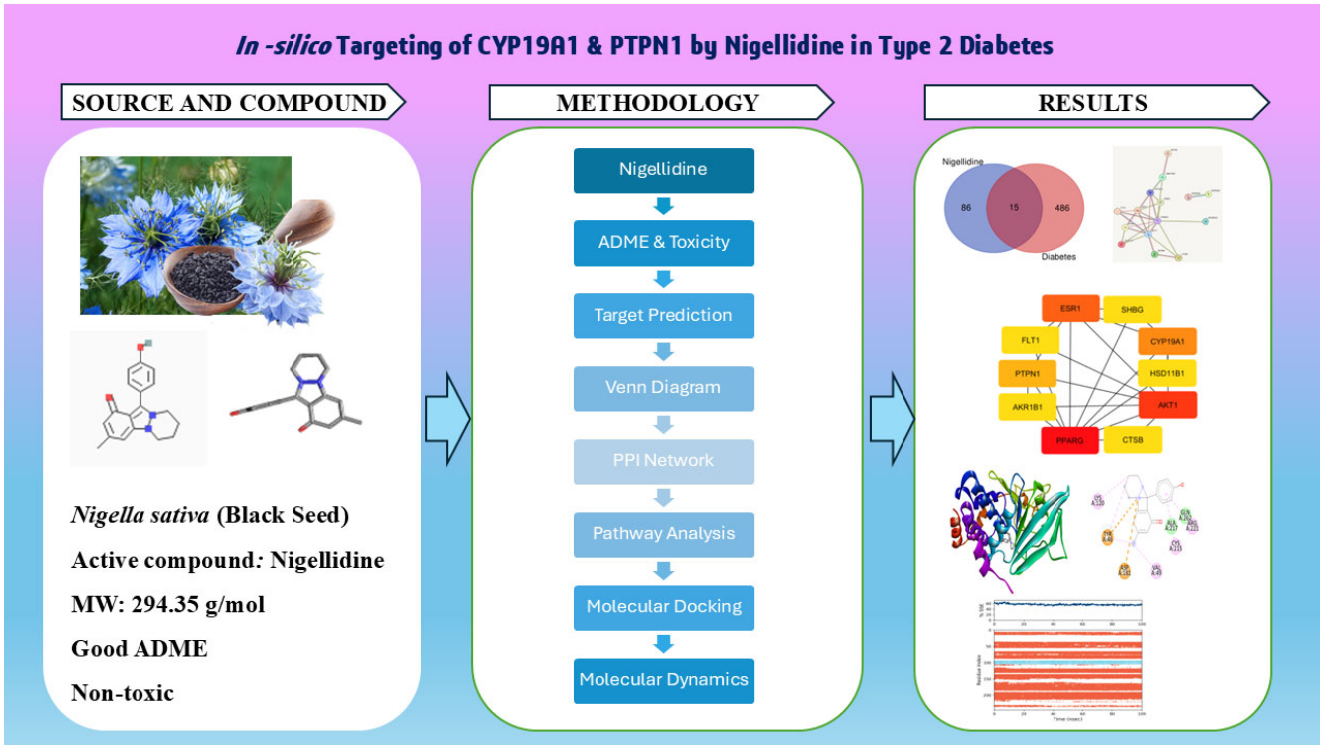


Graphical abstract

cent of the 80 per cent type 2 diabetes (T2D) cells is progressively diminished over time[6].T2D is a type of diabetes, which occurs almost in 90 per cent of all instances of diabetes, and is marked by insulin intolerance and progressive decline of the functions of the pancreatic T2D development is affected by a complex relationship between genetics and environment factors, obesity, physical inactivity and unhealthy diets are major factors leading to the development of T2D[7]. The trends experienced all over the world which include the rising cases of obesity, ageing and urbanization have been very important in contributing to the diabetes epidemic[8]. The current diabetes management is mainly focused on the attainment of glycemic control with respect to lifestyle modification, oral medications and insulin therapy[9]. Although these measures have positively impacted patient outcomes, they can be restricted by a variety of complications such as drug resistance, side effects, and disease progression that may force more complicated treatment plans as the disease progresses[10]. Moreover, the current medications primarily focus on the symptoms and do not treat the causes of the illness. In conjunction with the increased rate of diabetes, these restrictions have led to research into other forms of therapeutic interventions. The examination of natural compounds that possess anti-diabetic properties is also among the promising fields[11]. The strategy is inspired by the historical ways of practising traditional medicine where it has been using plant-based medicines to cure ailments that are akin to diabetes[12].

Nigellidine in *Nigella sativa*: A prospective natural product

Among the numerous plants that have been examined regarding their future potential in the treatment of diabetes, *Nigella sativa*, which is also referred to as black seed or black cumin, has gained immense popularity as a potential candidate. *N. sativa* is native to such areas as Southern Europe, North Africa, and Southwest Asia; it has a long history of use in traditional medicine systems, including Unani, Ayurveda, and Islamic medicine[13]. Its seeds as well as oil have been long used to cure a complex range of diseases, including diabetes, high blood pressure, inflammation, and infections[14]. Over the past few years, *Nigella sativa*, a natural product due to its use in diabetes treatment, has increased in popularity many times. Clinical and preclinical data are accumulating in support of its capability to control blood sugar levels, enhance insulin sensitivity and prevent diabetic complications[15]. These positive effects are to a large extent due to the rich character of

the plant in bioactive compounds rather than due to any one of the compounds, since they are rich with thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine, nigellicine, nigellidine, and alpha-hederin[16]. One of such molecules is Nigellidine, an alkaloid molecule, which recently appeared as a rather attractive molecule due to its possible anti-diabetic properties. Nigellidine was first isolated and identified in seeds of N. sativa in 1985[17]; it has been known to have broadly pharmacological activity in antioxidant, anti-inflammatory, and blood glucose-lowering (hypoglycemic) activities[18]. Of special interest is the possibility of Nigellidine being an anti-diabetic agent. Studies conducted so far have proposed that Nigellidine can have its hypoglycemic action in various ways, such as by means of increased secretion of insulin, enhanced insulin sensitivity, and modulation of critical enzymes implicated in the metabolism of glucose[13,21]. The existence of the metabolic and antidiabetic activity of *Nigella sativa* and constituent bioactive constituents has been documented in the past. Clinical and preclinical research has revealed that *N. sativa* supplementation can have a strong effect on reducing fasting blood glucose, HbA1c and lipid dysregulation and enhancing insulin sensitivity and the functionality[22]. It also has molecular and cellular metabolic effects, such as the ability to regulate glucose homeostasis as well as mitigate diabetes-related metabolic impairments[23]. *N. sativa* has also been reported to enhance glucose tolerance, reduce hepatic gluconeogenesis, normalize blood by lowering blood sugar levels and various other mechanisms that are considered multiple-factorial influences by which its bioactives produce antidiabetic effects[24].

Several biochemical, clinical, and bioinformatics studies have demonstrated the antidiabetic potential of *Nigella sativa* and its major phytoconstituents, reporting improvements in glycemic control, insulin sensitivity, and modulation of key metabolic pathways. However, most available evidence focuses on whole extracts or dominant constituents such as thymoquinone, while the molecular target networks of individual alkaloids remain insufficiently characterised. In particular, Nigellidine, an indazole alkaloid isolated from *N. sativa,* has shown promising pharmacological properties, yet its antidiabetic mechanisms are largely supported by prediction-based and pathway-level evidence rather than systematic target mapping. Therefore, the present study employed an integrative *in silico* approach combining ADME/Toxicity prediction, network pharmacology, protein–protein interaction analysis, pathway enrichment, molecular docking and dynamic simulation to identify potential molecular targets and signalling pathways through which Nigellidine may influence type 2 diabetes mellitus (T2DM). This study aims to provide a structured molecular framework to support future biochemical *in vitro* and *in vivo* validation studies.

Materials and Methods

Screening chemical structure and estimation of pharmacokinetic profile

The PubChem database, an open-access resource, provides critical information for drug discovery and chemical biology research. The canonical SMILES was retrieved from the PubChem database and used for pharmacokinetic evaluation through pkCSM and SwissADME[25]. These tools, which analyse data derived from SMILES strings, offer crucial insights into a compound's ADMET profiles. pkCSM, a widely recognised and reliable online platform, has been previously employed to investigate various physi-cochemical and pharmacokinetic properties of drug candidates[26].

Toxicity prediction

Toxicological testing is an essential part of drug development and safety supervision, and the aim of such testing is the reduction of the chances of adverse effect, *i.e*, organ damages or system failure. The toxicity of the chosen compounds in this study was assessed with the help of the OSIRIS software that compares the chemical structures with the data obtained at PubChem. The colour-coded scheme allows OSIRIS to stay ahead with a number of toxicological threats, such as: tumorigenicity, mutagenicity, irritation potential, and reproductive toxicity, as well as overall drug-likeness[27]. The tool also computes major parameters, which include Topological Polar Surface Area (TPSA), drug-likeness and a general drug score. According to the OSIRIS colour-coded system, the colour green depicts the very small risk of toxicity, the yellow shade indicates the moderate risk of toxicity, and the red colour implies the high risk of toxicity.

Prediction of the compound chosen and disease[28]

Nigellidine compound has been used in Swiss Target Prediction to come up with possible targets. The canonical SMILES of the compound were

recovered in the PubChem database and provided to the Swiss Target Prediction web server. The application makes use of 2D and 3D molecular similarity searches of a highly organized database of known ligand-target dimensions. The targeted proteins predicted were noted down. Parallel to that, the keyword Diabetes was employed in the DisGeNET database, where diabetes was retrieved as targets. The common targets between Nigellidine and Diabetes were calculated through the analysis of the common data sets and a Venn diagram was created with the help of online tool Bioinformatics and Evolutionary Genomics. The overlapping area of the diagram indicated that there were a number of common targets. Protein protein interaction network. The identified genes were further filtered to produce overlapping genes through Venn diagram with the remaining genes entered on the STRING web server to draw a protein-protein interaction (PPI) network with the species parameter set as Homo sapiens to filter off human-biased interactions. The data of the interactions in TSV format was subsequently imported into the Cytoscape where they were visualized and a network created. Degree values and network reliability was analyzed through the use of Network Analyzer of Cytoscape. To perform *in silico* further molecular docking, top 10 target genes (top degree) were picked.

Gene function and pathway enrichment analysis

Functional annotation was done on the top 10 target proteins found from the PPI network analysis by means of the DAVID database[29]. The biological function of Nigellidine's anti-diabetic targets was explored using GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis.

Compound-target molecular docking

The primary active compounds were retrieved from the PubChem database, and their chemical structures were initially drawn using ChemDraw. These structures were then imported into Chem3D Pro to generate three-dimensional conformations. To achieve stable configurations, conformational optimization was carried out using the "Calculation → MM2 → Minimize Energy → Run" function, and the optimized structures were subsequently saved in PDB format. The crystal structures of the target proteins were obtained from the Protein Data Bank (PDB) at http://www.rcsb.org/. Both the ligands (small molecules) and protein structures were then imported into AutoDock for molecular docking analysis. Among the generated docking poses, the one with the lowest binding energy was selected for further investigation[30]. The interactions between the bioactive compounds and the target proteins were finally visualized using BIOVIA Discovery Studio.

Molecular dynamics study

Molecular dynamics (MD) simulations were performed on the top-ranked docked complex of Nigellidine with CYP19A1 (PDB ID: 1XP1) using the Schrödinger Desmond 5.6.1 package on a Linux platform. The ligand topology files were generated using the PRODRG server. The system was prepared by applying the OPLS2005 force field, followed by solvation in an explicit TIP3P water model within a periodic boundary condition (PBC) box. To maintain electrical neutrality, appropriate Na^+ and Cl^- ions were added. Energy minimization was carried out for 2000 steps to remove steric clashes and stabilize the system. Subsequently, equilibration was performed under both NVT (constant volume and temperature) and NPT (constant pressure and temperature) ensembles using the Nose–Hoover thermostat and the Martyna–Tobias–Klein barostat at 300 K. Long-range electrostatic interactions were computed using the particle mesh Ewald (PME) method with a grid spacing of 0.8 Å. Following equilibration, a 100 ns production MD simulation was conducted using a leap-frog integrator with a time step of 2 fs, and trajectory data were recorded at 2 ps intervals. The stability and dynamic behaviour of the protein–ligand complex were evaluated through root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) analyses. Additionally, protein–ligand interactions throughout the simulation were examined using the Desmond simulation interaction diagram tool. This trajectory-based MD approach enabled a detailed assessment of conformational stability, molecular flexibility, and interaction persistence within the Nigellidine–CYP19A1 complex.

Results

Pharmacokinetic characteristics of Nigellidine

Nigellidine, a bioactive alkaloid from *Nigella sativa*, exhibits favorable pharmacological and molecular properties, suggesting its potential as a drug-like compound. It has a molecular formula of $C_{18}H_{18}N_2O_2$, a molecular weight of 294.35 g/mol in

(Table 1), and complies with drug-likeness criteria with only one rotatable bond, moderate hydrogen bonding potential (2 acceptors and 1 donor), and a low polar surface area (TPSA: 47.16 Å²), supporting good membrane permeability. Its lipophilicity is moderate, with a consensus Log Po/w of 2.82, and it is categorized as soluble to moderately soluble across different prediction models.

Pharmacokinetically, Nigellidine demonstrates high human intestinal absorption (95.37%) and Caco-2 permeability (log Papp 1.304), but low skin permeability. It is not a P-glycoprotein substrate but does inhibit P-gp I, which may affect drug efflux. Distribution parameters indicate moderate volume of distribution (log VDss 0.508) and low brain penetration (log BB -0.104), suggesting limited CNS exposure. It is metabolized as a substrate for CYP3A4 but not for CYP2D6 and inhibits CYP2C19 only. Excretion predictions show a total clearance rate of log 0.511 ml/min/kg and possible renal clearance through OCT2 interaction. Toxicity profiling revealed a non-mutagenic profile (negative Ames test), absence of cardiotoxicity (hERG I and II non-inhibitory), but flagged potential hepatotoxicity. It is not a skin sensitizer and shows moderate toxicity in aquatic models, as shown in (Table 2). Overall, these data support Nigellidine pharmacological promise while indicating the need for further investigation into its hepatic safety.

Table 1 — Pharmacological and molecular properties data of Nigellidine

Physicochemical Properties	
Formula	C18H18N2O2
Molecular weight	294.35 g/mol
Num. heavy atoms	22
Num. arom. heavy atoms	15
Fraction Csp3	0.28
Num. rotatable bonds	1
Num. H-bond acceptors	2
Num. H-bond donors	1
Molar Refractivity	88.65
TPSA	47.16 Å²
Lipophilicity	
Log Po/w (iLOGP)	2.57
Log Po/w (XLOGP3)	2.93
Log Po/w (WLOGP)	3.28
Log Po/w (MLOGP)	2.39
Log Po/w (SILICOS-IT)	2.93
Consensus Log Po/w	2.82
Water solubility	
Log *S* (ESOL)	-3.95
Solubility	3.31e-02 mg/ml ; 1.12e-04 mol/l
Class	Soluble
Log S (Ali)	-3.58
Solubility	7.71e-02 mg/ml ; 2.62e-04 mol/l
Class	Soluble
Log S (SILICOS-IT)	-4.6
Solubility	7.44e-03 mg/ml ; 2.53e-05 mol/l
Class	Moderately soluble

Toxicity prediction

Toxicological assessments of the experimental inhibitors were conducted using the OSIRIS property explorer prediction tool. Comprehensive evaluation and validation of each compound's toxicity profile are critical prerequisites for clinical development. As shown in the Table 3, Nigellidine exhibited a favourable safety profile, with no indications of tumorigenicity, mutagenicity, reproductive toxicity or irritancy. The majority of the candidate inhibitors also demonstrated low toxicity levels, underscoring their potential as safe therapeutic agents. Consistently positive toxicity predictions from OSIRIS further supported the overall safety of these compounds.

The TPSA is a key parameter influencing a molecule's ability to be absorbed and penetrate tissues. According to OSIRIS Property Explorer, the TPSA for Nigellidine was measured, supporting its potential for bioavailability. The compound showed an average drug-likeness score of -1.0. Additionally, the drug score, which integrates molecular weight, predicted toxicity, and lipophilicity (cLogP), serves as an important indicator of a compound's drug potential. Nigellidine, for example, achieved a drug score of 0.63, suggesting it meets the criteria for potential therapeutic application.

Target prediction

A comprehensive screening process initially yielded 499 diabetes-related target proteins by extracting data from the GeneCards and DisGeNET databases, with duplicate entries eliminated. In parallel, 100 potential target proteins of Nigellidine were predicted using the Swiss Target Prediction tool (Fig. 1). By intersecting these two datasets, 15 common target genes were identified, as shown in the Venn diagram (Fig. 2). These overlapping targets were then selected for further investigation through protein–protein interaction (PPI) analysis.

Input files

List names	Number of elements	Number of unique elements
Diabetes	678	499
Nigellidine	100	100
Overall number of unique elements		572

Table 2 — Pharmacokinetic parameters of Nigellidine

Property	Model Name	Predicted Value	Unit
Absorption	Water solubility	-3.651	Numeric (log mol/L)
Absorption	Caco2 permeability	1.304	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	95.368	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.916	Numeric (log Kp)
Absorption	P-glycoprotein substrate	No	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	0.508	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0.123	Numeric (Fu)
Distribution	BBB permeability	-0.104	Numeric (log BB)
Distribution	CNS permeability	-2.16	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	Yes	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	Yes	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	No	Categorical (Yes/No)
Excretion	Total Clearance	0.511	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	Yes	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	-0.425	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.423	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.081	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	Yes	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	1.437	Numeric (log ug/L)
Toxicity	Minnow toxicity	1.17	Numeric (log mM)

Table 3 — Assessing Nigellidine toxicity profile with Osiris

Parameters	Nigellidine (Scores)
Mutagenic	Green
Tumorigenic	Green
Irritant	Green
Reproductive effect	Green
TPSA	0.00
Drug Likeness	-1.0
Drug score	0.63

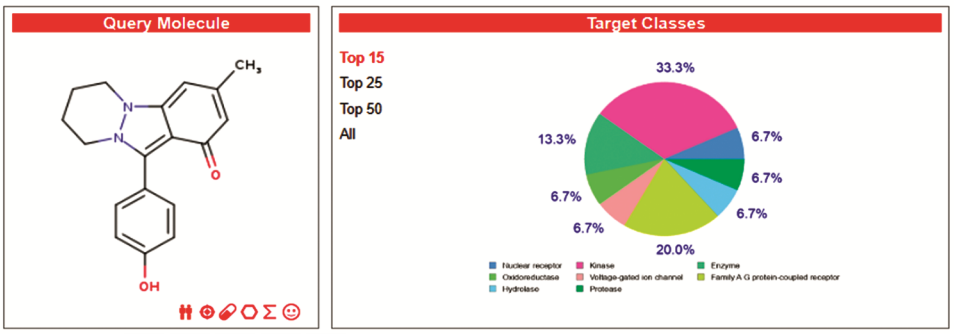
PPI network construction

The selected target genes were submitted to the STRING database, which generated a protein–protein interaction (PPI) network consisting of 15 nodes and 27 edges, illustrated in (Fig. 3). This network was then visualised and analyzed using Cytoscape version 3.9.1. Using the Network Analyzer tool, the degree value, an indicator of each node's connectivity within the network, was calculated. Based on these degree values, the top 10 hub genes (Fig. 4) identified were: PPARG (10), AKT1 (8), ESR1 (6), CYP19A1 (5), PTPN1 (4), HSD11B1 (3), AKR1B1 (3), FLT1 (3), SHBG (3), and CTSB (3). These hub genes represent key regulators within the PPI network, highlighting their critical roles in mediating various biological processes and signalling pathways.

Functional enrichment analysis

Gene Ontology (GO) enrichment analysis was performed, and the top ten terms for biological processes (BP), molecular functions (MF), and cellular components (CC) are illustrated in (Fig. 5). The biological process (BP) analysis highlighted involvement in the regulation of protein kinase B (Akt) activity, female pregnancy, tube diameter regulation, lipid catabolic processes, and vascular processes within the circulatory system.

In the analysis of molecular functions (MF), several critical activities related to drug-disease interactions were identified, such as protein phosphatase binding, steroid binding, estrogen receptor binding, nuclear receptor activity, and G-protein-coupled amine receptor activity. To further investigate the biological mechanisms associated with nigellidine, KEGG pathway enrichment analysis was conducted. The top ten enriched signalling pathways are shown in (Figs. 6 & 7). Notably, key pathways



Export results:

Show 15 entries Search:

Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Cathepsin S	CTSS	P25774	CHEMBL2954	Protease		13 / 0
Matrix metalloproteinase 14	MMP14	P50281	CHEMBL3869	Protease		7 / 0
Neuronal acetylcholine receptor; alpha4/beta2	CHRNA4 CHRNB2	P43681 P17787	CHEMBL1907589	Ligand-gated ion channel		9 / 0
Neuronal acetylcholine receptor; alpha3/beta4	CHRNA3 CHRNB4	P32297 P30926	CHEMBL1907594	Ligand-gated ion channel		2 / 0
Neuronal acetylcholine receptor protein alpha-7 subunit	CHRNA7	P36544	CHEMBL2492	Ligand-gated ion channel		6 / 0
Glycogen synthase kinase-3 beta	GSK3B	P49841	CHEMBL262	Kinase		80 / 0
Phosphodiesterase 4B	PDE4B	Q07343	CHEMBL275	Phosphodiesterase		29 / 0
Rho-associated protein kinase 2	ROCK2	O75116	CHEMBL2973	Kinase		56 / 0
C-C chemokine receptor type 3	CCR3	P51677	CHEMBL3473	Family A G protein-coupled receptor		5 / 0
Catechol O-methyltransferase	COMT	P21964	CHEMBL2023	Transferase		6 / 0

Fig. 1 — Swiss target prediction for Nigellidine

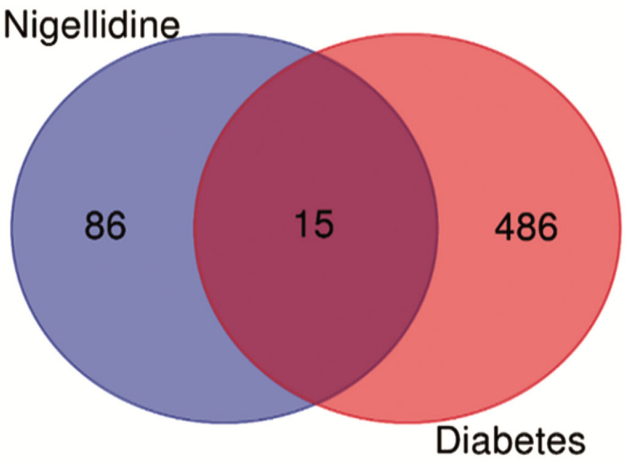


Fig. 2 — Venn diagram representing overlapping of diabetes targets

included insulin resistance, the cGMP–PKG signalling pathway, steroid hormone biosynthesis, and the prolactin signalling pathway.

Molecular docking

A comprehensive docking was carried out to assess the potential of Nigellidine to inhibit key targets

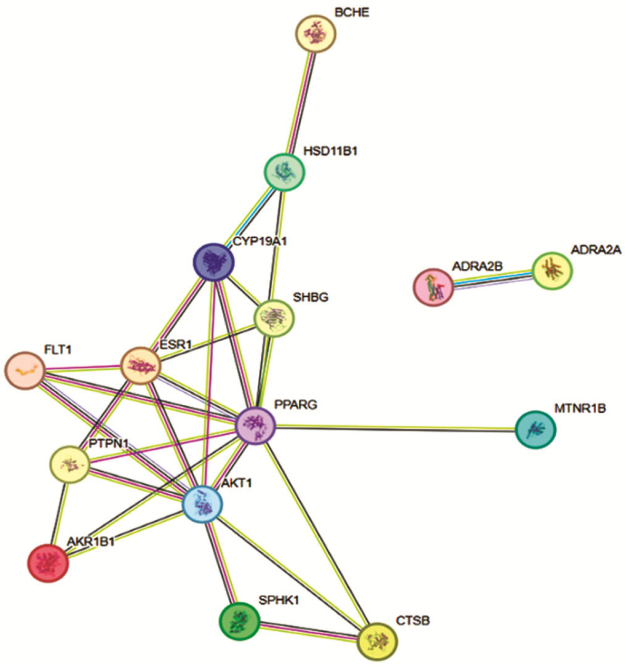


Fig. 3 — The protein–protein interaction (PPI) network of identified protein targets was retrieved from the STRING database and visualized using Cytoscape version 3.6.0.4

involved in hormonal regulation, oxidative stress, and insulin signalling pathways associated with diabetes. The predicted binding affinities for the selected targets ranged from –7.1 to –9.4 kcal/mol (Table 4). Several receptors exhibited strong binding affinities characterized by stable hydrogen bond interactions; however, only a limited number displayed notably favorable biochemical interactions within the protein's active site. To better understand these interactions, the binding modes of the targets with the highest docking scores were analyzed using Discovery Studio Visualizer. This analysis enabled the identification of specific amino acid residues involved in ligand–receptor binding at the active site (Fig. 8).

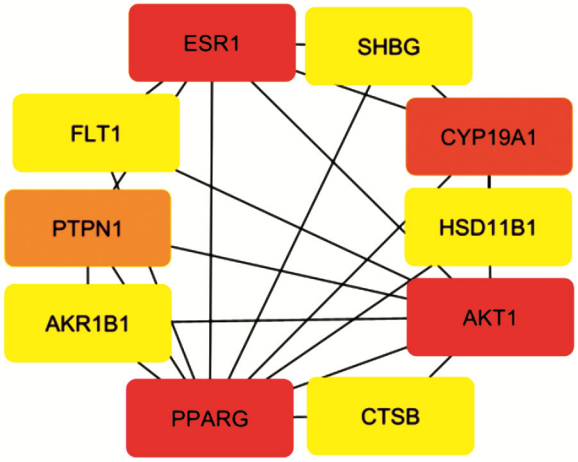


Fig. 4 — The top-ranked targets related to Nigellidine

Molecular dynamics study

The Desmond simulation software was used to perform a 100 ns molecular dynamics (MD) simulation for the Nigellidine–CYP19A1 (PDB ID: 1XP1) complex. While the ligand RMSD stayed

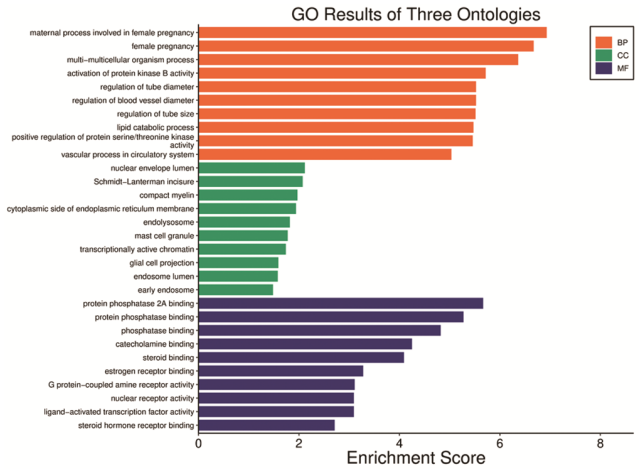


Fig. 5 — Gene ontology enrichment analysis (BP-biological process, CC-cellular components and MF molecular function)

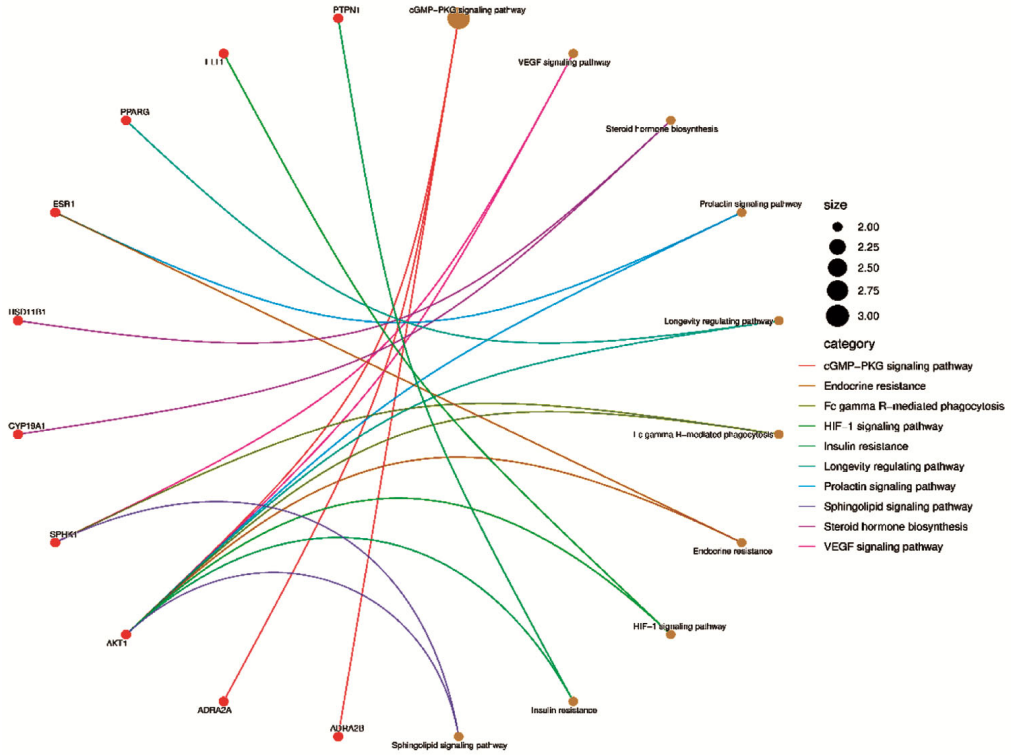


Fig. 6 — KEGG enrichment analyses

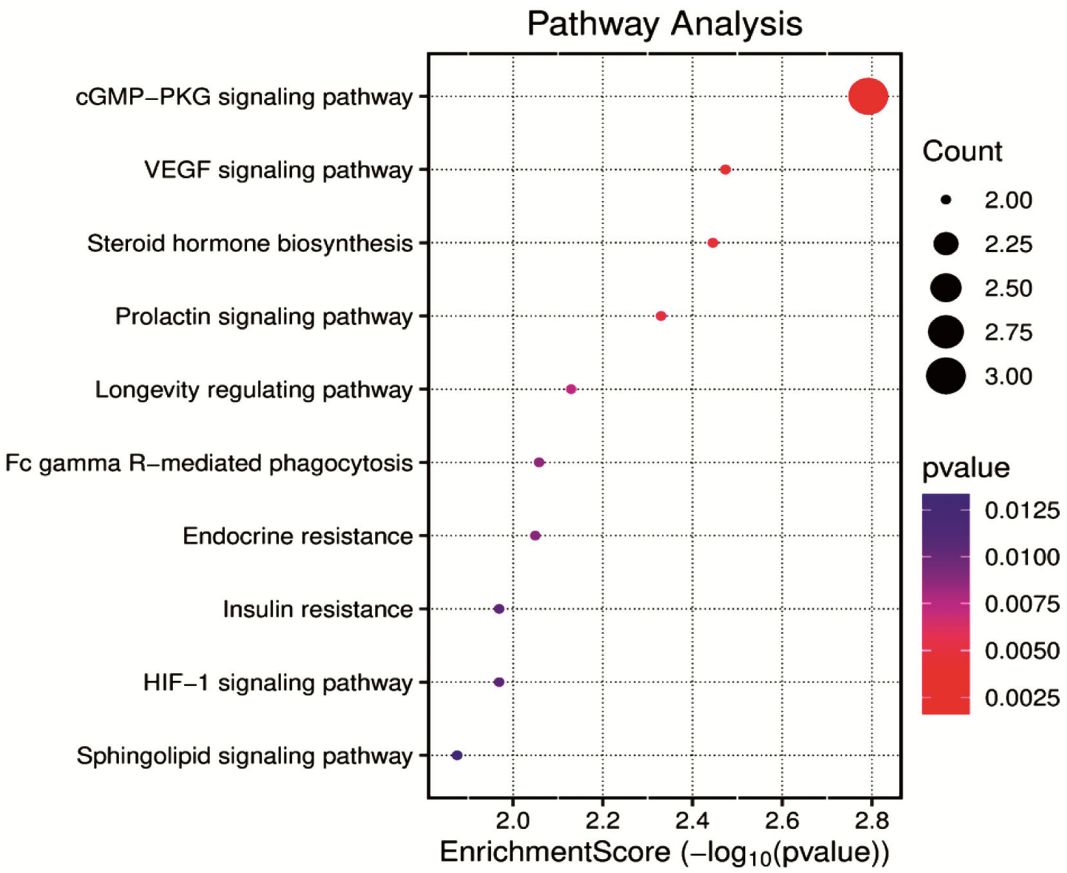


Fig. 7 — KEGG bubble chart showing 10 Pathway analysis

Table 4 — Docking assessment of Nigellidine on diabetes

Docking results of Nigellidine with key target molecules

Target protein	PDB ID	Attributes	Binding energy(kcal/mol)
ESR1	1KDM	X= -4.243727 Y= 39.095409 Z= 31.341727	-8.13
SHBG	1Q1M	X= 31.233388 Y= 27.549821 Z=23.626590	-8.18
CYP19A1	1XP1	X= 31.277647 Y= -1.499647 Z= 25.213324	-9.45
HSD11B1	2ILT	X= 59.219076 Y= 106.946418 Z= 45.137658	-8.84
AKT1	3EQM	X= 84.832541 Y= 53.101297 Z= 42.538176	-7.88
CTSB	3HNG	X= 4.505080 Y= 18.079200 Z= 33.902240	-7.21
PPARG	3O96	X= 8.373095 Y= -6.828310 Z= 12.622214	-8.31
AKR1B1	6MS7	X= 49.587025 Y= 10.677425 Z= 10.405075	-8.92
PTPN1	8FH6	X= 7.673203 Y= 35.975466 Z= 7.120653	-9.05
FLT1	8HE1	X= -5.701364 Y= 70.24572 Z= 117.176091	-7.16

within a similar range throughout the trajectory, the protein–ligand complex's root means square deviation (RMSD) profile (Fig. 9A) initially increased before stabilizing across the simulation. Residue flexibility varied across the protein structure, with comparatively smaller fluctuations seen at the active site regions, according to the root mean square fluctuation (RMSF) analysis (Fig. 9B). Changes in protein compactness during the simulation were reflected in the radius of gyration (Rg) plot (Fig. 9C), which showed slight fluctuations over time.

Hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges were observed at various time intervals in protein–ligand interaction analysis (Fig. 10A–C). The interaction timeline demonstrated prolonged contacts between Nigellidine and amino acid residues both inside and outside the binding pocket, while secondary structure element

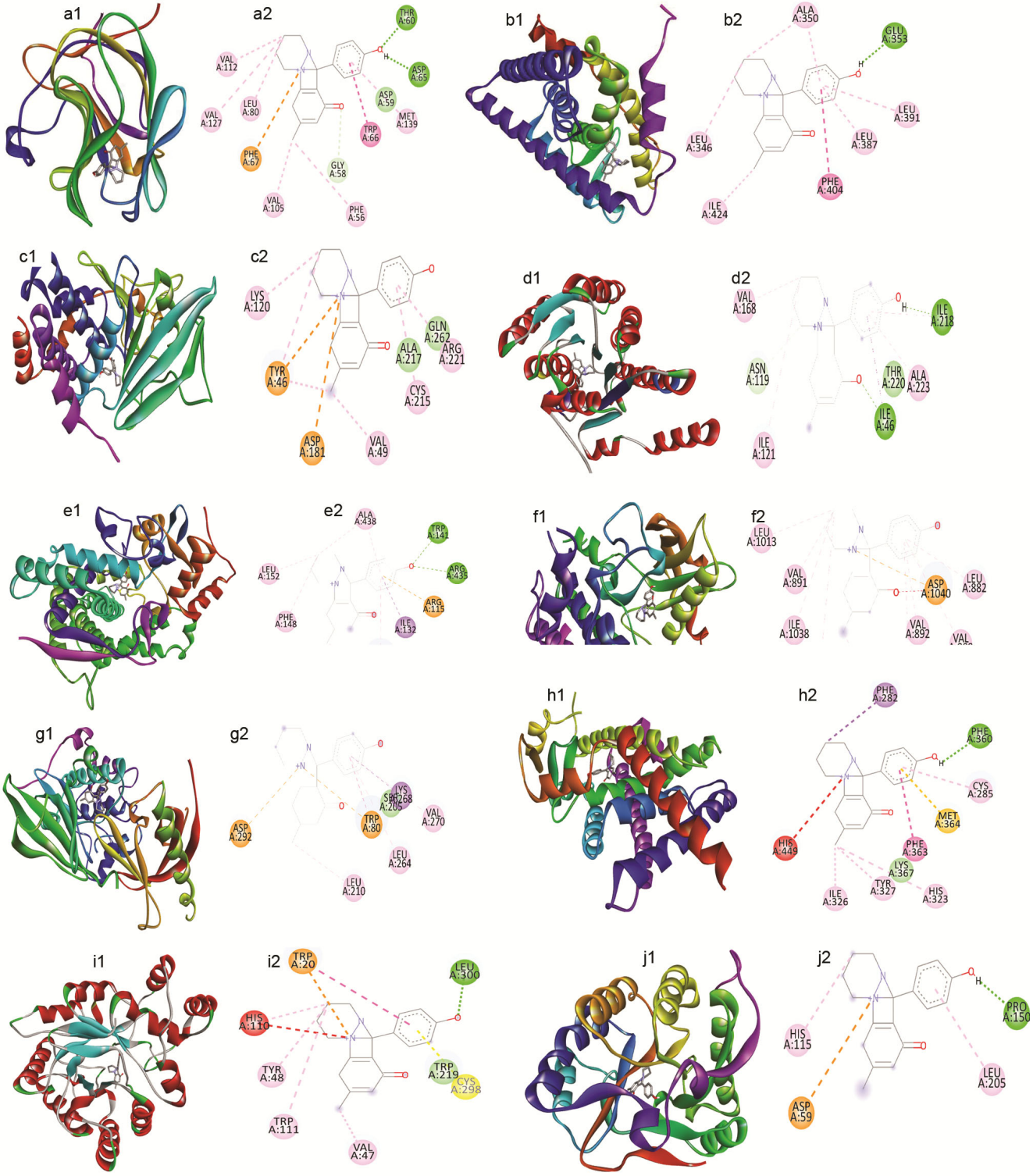


Fig. 8 — Visualizing the molecular docking of Nigellidine with diabetes-related targets. A1 and A2 is the 3D and 2D image of 1KDM, B1 and B2 is the 3D and 2D image of 1XP1, C1 and C2 is the 3D and 2D image of 1Q1M, D1 and D2 is the 3D and 2D image of 2ILT, E1 and E2 is the 3D and 2D image of 3EQM, F1 and F2 is the 3D and 2D image of 3HNG, G1 and G2 is the 3D and 2D image of 3O96, H1 and H2 is the 3D and 2D image of 6MS7, I1 and I2 is the 3D and 2D image of 8FH6, J1 and J2 is the 3D and 2D image of 8HE1

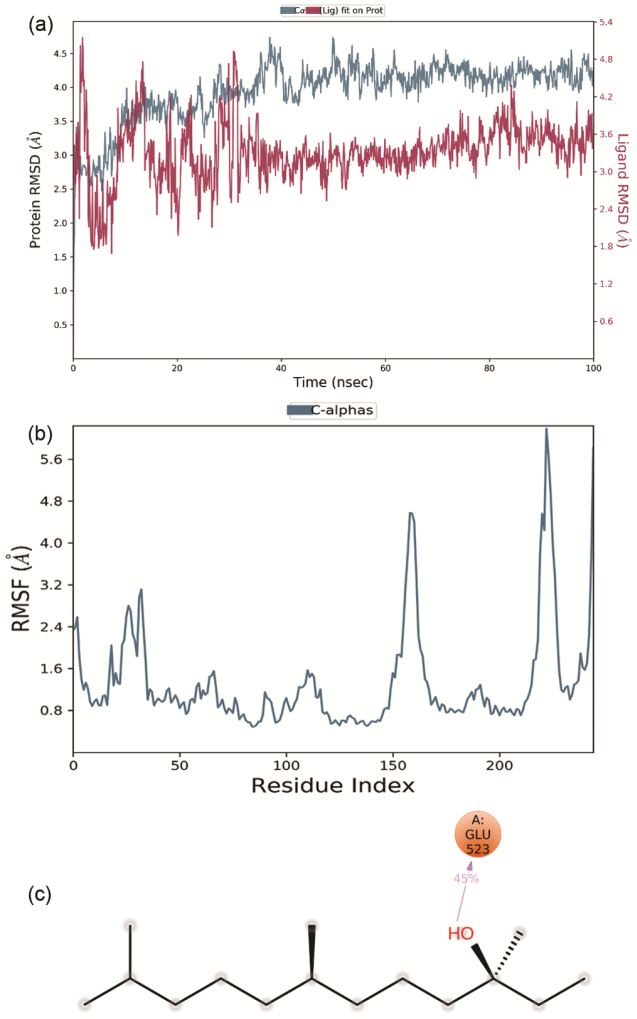


Fig. 9 — Graphical representation of (A) the ligand RMSD plot illustrating the variation of Nigellidine with 1XP1 during the molecular dynamics simulation, where green vertical lines indicate specific time points of ligand–residue interactions; (B) the RMSF plot showing the fluctuation of active site residues of 1XP1 in response to Nigellidine binding during the simulation; and (C) the radius of gyration (Rg) plot depicting changes in the compactness of the protein–ligand complex over the simulation period

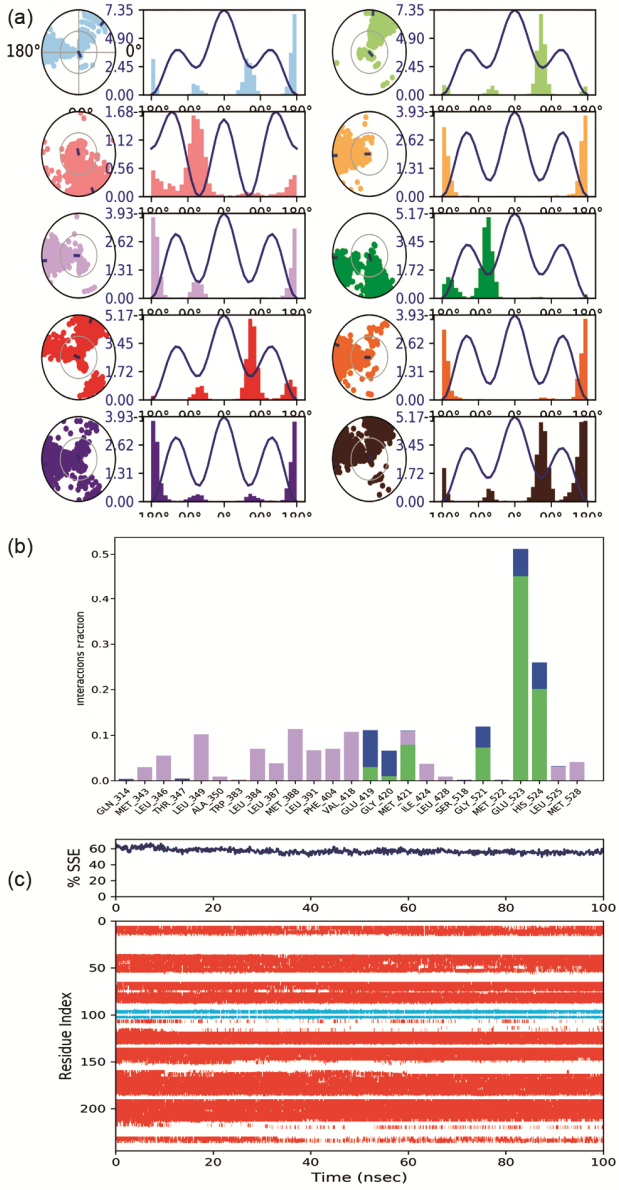


Fig. 10 — Graphical representation of the interactions between Nigellidine atoms and protein residues. (A) Visualization of ligand–residue interactions, showing contacts maintained for more than 30% of the simulation time. (B) Timeline-based stacked bar chart illustrating the interactions of Nigellidine with 1XP1 outside the active site pocket, including hydrogen bonds, hydrophobic interactions, ionic interactions, and water bridges. (C) Secondary structure element (SSE) composition plot depicting changes in residue assignments throughout the simulation period

(SSE) analysis showed changes in structural components over the course of the simulation.

Discussion

The present study provides a comprehensive *in silico* analysis integrating network pharmacology, ADME/toxicity prediction, molecular docking and dynamics simulation to evaluate the antidiabetic potential of nigellidine, an alkaloid from *Nigella sativa*. Nigellidine displayed favourable pharmacokinetic characteristics, including good oral absorption, blood-brain barrier permeability, and a non-toxic profile, aligning with previous findings that highlight the safety and drug-likeness of natural alkaloids in drug development[25].

The identification of 15 overlapping targets between Nigellidine and T2DM-related genes, including key hub proteins such as CYP19A1, PTPN1, and HSD11B1, underlines its multi-targeted mechanism. These proteins are intricately linked to insulin signalling,

glucose metabolism, and hormonal regulation, central components in the pathogenesis of T2DM.

Recent network pharmacology and molecular analyses have highlighted PTPN1 as a negative regulator of insulin-PI3K/Akt signalling, where modulation may improve insulin sensitivity and glucose utilization[26,32]. HSD11B1 plays a role in local cortisol regeneration in adipose and liver tissue, with inhibition reducing gluconeogenesis and insulin resistance, supported by both mechanistic reviews and *in silico* inhibitor screening [33]. CYP19A1, involved in estrogen biosynthesis, also emerged in network studies as influencing metabolic inflammation and steroid-mediated insulin sensitivity regulation, reflecting its broader contribution to glucose homeostasis[34].

Functional enrichment analysis revealed the involvement of Nigellidine-targeted proteins in critical pathways such as PI3K-Akt signalling**,** AGE-RAGE signalling**,** and insulin resistance, corroborating existing literature that identifies these pathways as central to diabetic complications[10,11]. The significant docking affinities observed between Nigellidine and multiple targets (e.g, -9.45 kcal/mol for CYP19A1) suggest a high likelihood of stable binding and functional modulation, further supporting its role as a potential antidiabetic lead molecule.

The molecular dynamics simulation further substantiated the docking outcomes by confirming the structural stability and dynamic consistency of the Nigellidine–CYP19A1 complex. The RMSD values were determined based on a simulation timescale of 100 ns. The RMSD trajectory of the complex remained within an acceptable deviation range, indicating that the ligand retained a stable conformation within the binding pocket without significant displacement during the simulation period. Similarly, RMSF analysis demonstrated low fluctuation values at key active-site residues, suggesting reduced structural flexibility and strong stabilization of the binding region in the presence of Nigellidine. The stability of the radius of gyration values indicated preserved compactness and overall structural integrity of the complex. Interaction analysis revealed persistent hydrogen bonds, hydrophobic interactions, and water bridges, many of which were maintained for a considerable portion of the simulation duration, indicating sustained binding affinity. Collectively, these observations reinforce that Nigellidine forms a dynamically stable and energetically favorable complex with CYP19A1, thereby supporting its potential inhibitory relevance in diabetes-associated molecular regulation.

Nigellidine is a promising chemical with complementary properties that may be used to create a multi-target treatment strategy for type 2 diabetes. To validate these *in silico* results and evaluate their therapeutic efficacy *in vivo*, experimental research is required. The existence of several substances that target various pathways indicates a complex therapeutic strategy, which makes these substances attractive options for more research in the treatment of diabetes.

Conclusion

It was systematic research whereby the antidiabetic properties of Nigellidine, a bioactive alkaloid of *Nigella sativa*, were investigated using an integrative network pharmacology and molecular docking system. These data indicate that Nigellidine has desirable drug-like characteristics and multi-target action through interactions with essential proteins involved in the pathophysiology of diabetes. The analysis of the protein-protein interaction revealed key targets that include CYP19A1, PTPN1, HSD11B1, and AKT1 that relate to insulin signalling, glucose metabolism, and inflammation. Its role in functional enrichment in other pathways known to be significant in diabetic complications was also illustrated by its presence in the PI3K-Akt and AGE-RAGE signalling pathways. These key targets were found to bind with a strong binding affinity through molecular docking, thus supporting their potential applicational role. This was proved by the molecular dynamics simulation that Nigellidine binds to CYP19A1, forming a stable and dynamically consistent complex, which validates its sound binding and functional usefulness. Taken together, the findings back up an effect of Nigellidine as a multi-target antidiabetic agent, and thereby offer a molecular rationale to its customary application in diabetes treatment. These findings provide the foundation of *in vitro* and *in vivo* investigations to come and clinical validation as well as point out the possibilities of natural products in the creation of safer and more efficient interventions in diabetes and its related complications.

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Conflict of interest

Both the authors declare no conflicts of interest.

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