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ORIGINAL ARTICLE

A Bioinformatics-based Investigation to Screen and Analyze the Bioactivity of *Piper longum* Linn. Compounds as a Ground-breaking Hostile to Antidiabetic Activity

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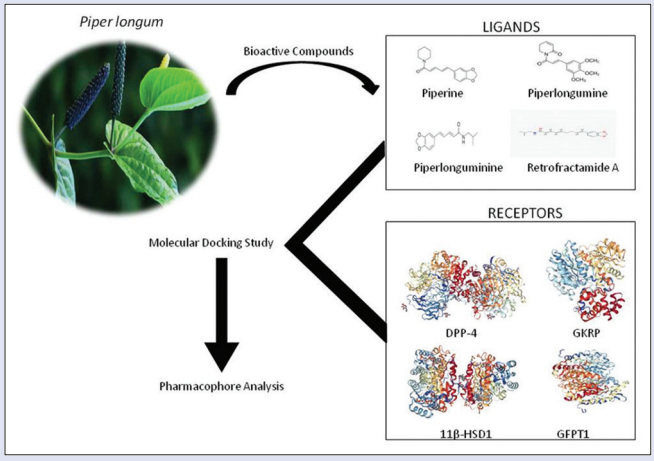
ABSTRACT

As of today, the utilization of herbal medicines has taken up the pace in treating diseases. This is due to the fact that they have lower risk of adverse reactions. Numerous plants are being used traditionally to treat various dreadful diseases including diabetes. *Piper longum* is one of the major and important medicinal plants in various systems of medicine, including the Ayurvedic system of medicine. Among the major bioactive compounds found in this plant, few compounds, viz., piperine, piperlongumine, piperlonguminine, and retrofractamide A, have been selected for studying the effectiveness on antidiabetic activity. An *in silico* approach was utilized to observe the major phytochemical properties and interaction studies of the constituents of *P. longum*, and finally, a pharmacophore investigation was carried out. In this study, we have observed the interaction of the four bioactive compounds taken from *P. longum* with the receptors via molecular docking technique. The binding of the ligands firmly with the receptors confirmed the fact that piperine, piperlongumine, piperlonguminine, and retrofractamide A act as inhibitors for dipeptidyl peptidase-4, GKRP, 11β-hydroxysteroid dehydrogenase type 1, glutamine-fructose-6-phosphate transaminase 1, and protein tyrosine phosphatase 1B, which encourage the glucose digestion and increment insulin affectability. The information acquired from this investigation might be taken further for *in vitro* examinations, which may, in the long run, be useful in recognizable proof of novel and successful particles. The outcomes acquired from this examination might provide strong understanding in the utility of phytochemicals against diabetes.

Key words: Antidiabetic, bioactive compounds, molecular docking, pharmacophore, *Piper longum*



Abbreviations used: DPP-4: Dipeptidyl peptidase inhibitor 4; GKRP: Glucokinase regulatory protein; 11β-HSD1: 11β-Hydroxysteroid dehydrogenase type 1; GFPT1: Glutamine-fructose-6-phosphate transaminase 1; PTB1B: Protein tyrosine phosphatase 1B.

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INTRODUCTION

Piper longum Linn., additionally called as long pepper, is one of the regular culinary herbs, which has been widely utilized as a constituent in different indigenous drugs. This long pepper has bioavailability upgrading action for some dietary substances and a few medications.[1] The pharmacological properties of this plant additionally incorporate the antioxidant, mitigating, hostile to hyperlipidemic, against corpulence and hepatoprotective.[2] Piperine, an alkaloid from *Piper nigrum* and *P. longum*, is a known inhibitor of different proteins in charge of biotransformation of medications. By restraining the digestion of medications, piperine improves the bioavailability of medications[3] which are hostile to diabetic action of the roots as likewise has been reported. Piperine in the blend with a sub-remedial portion of metformin has been reported for lowering blood glucose levels when contrasted with control gathering and furthermore demonstrated the more prominent bringing down of blood glucose[4] by other compounds alone or in a combination of piperine, piperlongumine, piperlonguminine, and retrofractamide A are recognized in *P. longum*. The detailed pharmacological exercises of piperlongumine incorporate cytotoxic, genotoxic, tumor enhancement, anti-angiogenic, anti-metastatic, anti-platelet conglomeration, antinociceptive, anxiolytic, energizer, prone to atherosclerotic, antidiabetic, antibacterial, hostile to contagious, leishmanicidal, trypanocidal, and schistosomicidal exercises.[5] Piperlonguminine and retrofractamide A which essentially expanded the measure of adiponectin discharged

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into the medium just as the take-up of 2-deoxyglucose (DG) into the cells.[6]

Diabetes mellitus (DM) is a heterogeneous gathering of metabolic infection described by constant hyperglycemia caused due to diminished insulin emission as well as expanded insulin obstruction.[7] As opposed to type 1 diabetes that displays hyperglycemia and hypoinsulinemia, type 2 diabetes frequently show hyperglycemia and hyperinsulinemia.[8] The mix of decreased glucose digestion and insulin obstruction prompts steady hyperglycemia in the fasting just as in the postprandial state.[9] Patients with type 1 diabetes suffer from insulin insufficiency, inferable from the pancreatic β-cell disappointment, and insulin is an essential and viable treatment to bring down hyperglycemia.[10] The element of type 2 DM (T2DM) is fractional or deficient in utilizing insulin even though synthetic insulin is accessible.[11]

Numerous endeavors to make sense of the compelling medications for T2DM have been expanded. In the present decade, the pervasiveness of diabetes and its repeat and extreme reactions of chemotherapeutic medicines have prompted the resurgence of interests in the utilization of homegrown arrangements as increasingly viable medications with less or no symptoms. At present, inquire about on connection between hostile to diabetic bioactive mixes and T2DM has been very much distributed and archived.[12]

In light of these confirmations, the goal of this examination is to distinguish antidiabetic properties of the selected bioactive compounds piperine, piperlongumine, piperlonguminine, and retrofractamide A recognized in *P. longum* by utilizing them as ligand against the five focused proteins: 11-β hydroxysteroid dehydrogenase type 1 (11β-HSD1), glutamine-fructose-6-phosphate transaminase 1 (GFPT1), protein-tyrosine phosphatase 1B, dipeptidyl peptidase-4 (DPP-4), and glucokinase administrative protein.

The receptor focuses for T2DM as detailed by numerous researchers to date are glycogen phosphorylase, protein tyrosine phosphate 1-beta, DPP-4, glucokinase, peroxisome proliferator enacted receptor-gamma, aldose reductase, insulin receptor, etc.[12] 11β-HSD1 restraint is an enticing objective for the treatment of glucocorticoid-related infections, particularly T2DM. In fat tissue, inordinate cortisol generation through 11β-HSD1 movement has been linked with pathogenesis of T2DM and heftiness.[13] Glutamine-fructose-6-phosphate amidotransferase (GFAT) or GFPT1 is the first and rate constraining protein of the hexosamine pathway. GFAT controls the motion of glucose into the hexosamine pathway and catalyzes the development of glucosamine-6-phosphate. Expanded GFPT1 or GFAT movement was connected to postprandial hyperglycemia, and this may speak to an early biochemical distortion in the normal history of T2DM.[14] The hexosamine biosynthetic pathway is an elective pathway of glucose digestion that has, as of late, been involved in the pathogenesis of diabetic nephropathy.[15] Therefore, this compound is a helpful objective against T2DM. Protein tyrosine phosphatase 1B (PTP1B) is a key individual from the family, a negative controller in insulin signal transduction, and a potential objective for the treatment of T2DM.[16] DPP-4 inactivates glucagon-like peptide-1 which invigorates insulin emission and stifles glucagon discharge. Hence, DPP-4 inhibitors can go about as potential medication contender for T2DM. DPP-4 inhibitors, which have been generally utilized as remarkable blood glucose-subordinate enemy of diabetic specialists for patients with T2DM, show guarantee.[17] GKRP (glucokinase administrative protein) shapes an inhibitory complex with glucokinase, the catalyst in charge of directing the take-up and capacity of dietary glucose.[18] Along these lines, ligands that can go about as an inhibitor of GKRP will encourage the digestion of glucose in this manner bringing down hyperglycemia.

Docking procedure plans to foresee the exploratory restricting modes and affinities of little particles inside the coupling site of specific receptor targets and is presently utilized a standard computational instrument in medication structure for lead compound advancement and virtual screening concentrates to discover novel naturally available compounds.[19] The docking device utilizes various calculations; the most prominently utilized is a genetic algorithm. Pharmacophore investigation is a clarification venture for docking results: low or high restricting liking of ligand to receptors. The target of this investigation was to distinguish the antidiabetic action of the four bioactive compounds present in *P. longum* as they have been contemplated *in vivo* and *in vitro* to have hostile to diabetic properties where the insulin-flagging and -related falls alongside protein articulation and action or potentially phosphorylation studies were considered yet did not have an *in silico* approach. Subsequently, the examination concentrates more on the *in silico* to check the setup certainty all the more absolutely.

METHODOLOGY

Receptor preparation

Three-dimensional (3D) structure of 11β-HSD1, GFPT1, protein tyrosine phosphatase 1B (PTB1B), DPP-4, and GKRP were taken from Protein Data Bank (PDB)[20] as follows: 11β-HSD1 (PDB code 1XU7), GFPT1 (PDB code 2V4M), PTB1B (PDB code 3SME), DPP-4 (PDB code 1J2E), and GKRP (PDB code 4BBA). UCSF Chimera; Chimera 1.13.1rc[21] was used for visualization.

Bioactive compound preparation

All the bioactive compounds were downloaded from ChemSpider:[22] Retrofractamide A (CID: 9188045), piperine (CID: 553590), piperlongumine (CID: 553441), and piperlonguminine (CID: 4478660). The 2D representations of all bioactive compounds were copied as SMILES format and converted to PDB format with 3D coordinates using Open Babel.[23] UCSF Chimera 1.13.1rc was used for visualization. Ligands were checked for torsion count to detect currently active bonds with default settings.

Physiochemical properties

The physicochemical properties of the four bioactive compounds had been retrieved from ChemSpider in the form of molecular formula, average mass, boiling point, hydrogen bond donor, hydrogen bond acceptor, freely rotating bonds, polar surface area, molar refractivity, monoisotopic mass, rule of five violations, and ACD/logP. Table 1 represents the physicochemical properties.

Bioactivity score

The bioactivity scores of all bioactive compounds were also calculated for different parameters, G protein-coupled receptor ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor, and nuclear receptor ligand activity. As we know for metal heterocyclic complexes, if the bioactivity score is more than 0.00, then the complex is active; however, if it is between −0.50 and 0.00, then the complex is moderately active, and if the complex has −0.50, then it is inactive. The potential bioactivity scores of all ligands are given in Table 2, which indicates that all ligands show those properties which are required for the characteristics of ligand for acting as a drug. The data are obtained from the Molinspiration tool (predict bioactivity module).[24]

Virtual screening by Lipinski's rule of five

Lipinski's rule of five[25] helps in distinguishing between drug-like and nondrug-like molecules. It predicts high probability of success or failure due to drug-likeness for molecules complying with two or more of the rules: molecular mass <500 Dalton, high lipophilicity

Table 1: Chemical and physical properties: Piperine, piperlongumine, piperlonguminine, and retrofractamide A

Parameters	Bioactive compounds			
	Piperine	Retrofractamide A	Piperlongumine	Piperlonguminine
ChemSpider CID	553590	9188045	553441	4478660
Molecular formula	$C_{17}H_{19}NO_3$	$C_{20}H_{25}NO_3$	$C_{17}H_{19}NO_5$	$C_{16}H_{19}NO_3$
Average mass (Da)	285.338	327.417	317.336	273.327
Boiling point (mmHg)	498	562	475.6	476.9
Hydrogen bond donors	0	1	0	1
Hydrogen bond acceptors	4	4	6	4
Freely rotatable bonds	3	8	5	5
Polar surface area (A^2)	39	48	65	48
Monoisotopic mass (Da)	285.136505	327.183441	317.126312	273.136505
Molar refractivity (cm^3)	82.1	98.3	86.4	79.7
Rule of 5 violation (s)	0	0	0	0

Table 2: Bioactivity scores of all bioactive compounds obtained from Molinspiration

Ligands	GPCR ligand	Ion channel Modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Piperine	0.15	-0.18	-0.13	-0.13	-0.10	0.04
Retrofractamide A	0.22	0.02	-0.08	0.09	0.16	0.12
Piperlongumine	0.21	-0.03	-0.07	-0.08	-0.05	0.08
Piperlonguminine	0.04	-0.19	-0.30	-0.22	-0.06	-0.00

GPCR: G protein-coupled receptor

(expressed as LogP <5), <5 hydrogen bond donors, less than 10 hydrogen bond acceptors, and molar refractivity between 40 and 130. This rule had been verified using the SCFBio drug designing tool named Sanjeevini.[26]

Molecular docking

Molecular docking studies were performed using offline AutoDockTools-1.5.6[27] on Windows platform. The AutoDockTools was used with the help of the MGL tools available. Lamarckian genetic algorithm was used to dock the four bioactive compounds: piperine, piperlongumine, piperlonguminine, and retrofractamide A against 11β-HSD1, GFPT1, protein-tyrosine phosphatase 1B, DPP-4, and glucokinase regulatory protein. The receptors were kept as a macromolecule and have been kept as rigid, and the ligands were kept flexible. The grid was enacted in such a way that the macromolecule and the ligands were submerged within the given grid parameters for all of the four ligands against the macromolecule.

Pharmacophore analysis

Pharmacophore is an explanation step for the molecular docking result: low or high binding affinity of the ligand to receptors. This part of the process was carried out using the pharmacophore tool included in LigandScout.[28] The program showed us the 2D and 3D structure of position and interaction of ligand in the binding pocket of the receptor. From these 2D figures, some types of the bond were identified by color. Three features namely hydrogen bond donor, hydrogen bond acceptor, aromatic interactions, and hydrophobic interactions were labeled.

RESULTS

Physiochemical properties

The physiochemical properties of the four bioactive compounds are referenced in Table 1, which were all recovered from ChemSpider. Among the bioactive compounds, piperlongumine holds the maximum number of hydrogen bond acceptors and also the polar surface area of piperlongumine is the highest among the others. In terms of the monoisotopic mass, retrofractamide A and piperlongumine have higher masses than piperine and piperlonguminine. All the four bioactive compounds, namely piperine, piperlongumine, retrofractamide A, and piperlonguminine, do not violate the rule of five.

Bioactivity score

The bioactivity scores, which incorporate the GCPR ligand, particle channel modulator, kinase inhibitor, atomic receptor ligand, protease inhibitor, and compound inhibitor, are referenced in Table 2.

Virtual screening by Lipinski's standard of five

The outcomes demonstrated that each of the four ligands passed Lipinski's standard of five and can go about as medication. The scores of all ligands are given in Table 3. All the four ligands taken show subatomic mass under 500 Da, high lipophilicity (communicated as LogP) under 5, under 5 hydrogen security contributors, under 10 hydrogen security acceptors, and molar refractivity between 40 and 130.

Subatomic docking

Subatomic docking of each of the five objective proteins or receptors 11β-HSD1, GFPT1, protein-tyrosine phosphatase 1B, DPP-4, and glucokinase administrative protein, with every one of the four ligands, piperine, retrofractamide A, piperlongumine, and piperlonguminine, was performed utilizing AutoDockTools,[29] and 10 docking adaptations were created for every ligand. In any case, the conformer with the most astounding restricting vitality and least inhibition constant (Ki) has been accounted for. The parameters which have been accounted for in Table 4 are restricting vitality, ligand effectiveness, hindrance consistent, intermolecular vitality, van der Waals HB-desolvation vitality, all-out interior vitality, torsional vitality, unbound vitality, clRMS, and refRMS. Therefore, the outcomes got in this examination plainly shows inhibition of receptors by the ligands, piperine, piperlongumine, piperlonguminine, and retrofractamide A (acting as inhibitors), which recommends that these inhibitors can follow up on DPP-4, GKRP, 11β-HSD1, GFPT1, and PTB1B, which will encourage glucose digestion and increment insulin affectability. The most

Table 3: Validation of Lipinski's rule of 5

Ligand	Mass	Hydrogen		LogP	Molar refractivity
		Bond donor	Bond acceptor		
Piperine	285	0	4	2.997200	81.16
Retrofractamide A	327	1	4	4.093	96.96
Piperlongumine	317	0	6	2.040700	85.60
Piperlonguminine	273	1	4	2.75	78.58

The validation was done with the help of SCFBio drug designing tool, Sanjeevini

astounding restricting vitality was seen in piperine with −8.96 kcal/mol pursued by retrofractamide A with −7.78 kcal/mol, piperlonguminine with −7.33 kcal/mol, and piperlongumine with −6.57 kcal/mol separately. Piperine additionally demonstrated the least hindrance steady (Ki) 0.429 μM showing the most grounded inhibitor of DPP-4. The docking adaptations which are spoken to in Figure 1 indicate piperine and retrofractamide A complexed with DPP-4 and GKRP. This may be represented by the way that the bioactive mixes of *P. longum* have hostile to diabetic potential. Thus, from the coupling energies of all the four bioactive metabolites, piperine demonstrates the most astounding restricting fondness pursued by retrofractamide A, piperlonguminine, and piperlongumine. The chart of restricting vitality versus compliances has been created for all the four bioactive mixes and is spoken to in Figure 2. DPP-4 mainly interacts with ligands via hydrophobic bonds and hydrogen bond donor; GKRP interacts with mentioned ligands via hydrogen bond donor, hydrophobic bonds, and also hydrogen bond acceptors; 11β-HSD1 interacts with the ligands via hydrophobic bonds and hydrogen bond donor; GFPT1 and PTB1B interacts with the ligands via hydrophobic bonds, hydrogen bond acceptor along with aromatic bonds.

Inhibition constant (Ki)

The subatomic docking results demonstrate the restraint steady, which gives us an impression of the quality of docked ligand to go about as an inhibitor of the receptor. Piperine demonstrated the least hindrance steady 0.429 μM against DPP-4 trailed by retrofractamide A with 1.97 μM against GKRP (glucokinase administrative protein). The least hindrance steady of piperine and retrofractamide A approves their job as potential medication up-and-comer since restraints of DPP-4 and GKRP assume a job in glucose digestion consequently bringing down hyperglycemia. Furthermore, piperlongumine and piperlonguminine are the potential drug candidates, but the potential in piperine and retrofractamide A is more than the other two.

Pharmacophore investigation

This progression clarifies the outcomes acquired from atomic docking. It likewise clarifies the high- and low-restricting fondness of ligands to receptors. Two ligands piperine and retrofractamide A and their collaboration with DPP-4 and GKRP were chosen for pharmacophore investigation as they indicated generally excellent docking scores. Pharmacophore examination appeared in Figure 3 indicates two highlights in particular hydrogen bond contributor and hydrophobic connections. The outcomes unmistakably show the amino corrosive positions and their connections in this manner further approving the coupling partiality. Figure 3 speaks to the pharmacophore investigation of piperine and retrofractamide A. Table 5 demonstrates the amino corrosive positions and sort of collaborations of every bioactive compound.

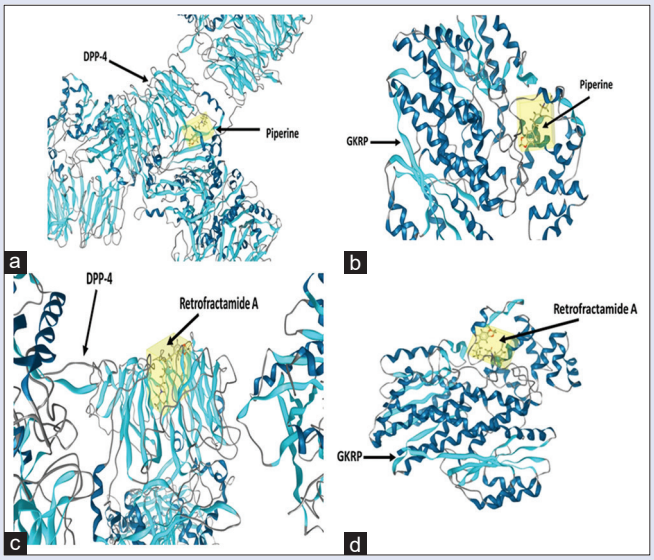

Figure 1: Docked complexes of (a) piperine with dipeptidyl peptidase-4, (b) piperine with GKRP, (c) retrofractamide A with dipeptidyl peptidase-4, and (d) retrofractamide A with GKRP. The above complexes were prepared in AutoDock using Lamarckian genetic algorithm and were visualized in LigandScout

DISCUSSION

DM is one of the normal metabolic issues securing 2.8% of the total populace and is foreseen to cross 5.4% constantly by 2025.[1] Hyperglycemia is regular in both T1DM and T2DM. T2DM is portrayed by insulin obstruction and weakened pancreatic β-cell work at the conclusion and dynamic β-cell brokenness after some time.[30] Both T1DM and T2DM influence a large number of individuals all around the globe. Natural drugs have turned out to be unequivocally favored treatment to lessen the negative effect of DM and its extreme complexities because of lesser symptoms and minimal effort. *In vivo* and *in vitro* investigations of some major bioactive compounds have entrenched the connection of the bioactive compounds and their effort in bringing down hyperglycemia, along these lines being powerful against DM. *In vitro* and *in vivo* proof incorporates the impact on insulin-flagging and related falls in β-cells and so on. Furthermore, it influences as far as quality interpretation, protein articulation, movement, as well as phosphorylation.[31] In any case, it comes up short on an *in silico* way to deal with distinguish major bioactive mixes and build up their job in bringing down hyperglycemia and improving insulin affectability. This can be accomplished by examining the association of the receptors and ligands employing subatomic docking and pharmacophore investigation draws near. As of late, a solid enemy of the hyperglycemic impact of *P. longum* on mice models has been accounted for, yet the activity instrument of its bioactive compounds has stayed obscure. The nearness of many plant optional metabolites makes *P. longum* a remedial center

Table 4: Depicting the various parameters of the docked receptors with various ligands (piperine, piperlongumine, piperlonguminine and retrofractamide A)

Receptor	Ligand	Binding energy (Kcal/mol)	Ligand efficiency	Inhibition constant (μmol)	Intermol energy	van der Waals HB-desolving energy	Electrostatic energy	Total internal energy	Torsional energy	Unbound energy	cIRMS	refRMS
DPP-4	Piperine	-8.96	-0.41	0.429	-9.58	-9.41	-0.17	-0.43	0.89	-0.43	0.0	4.93
	Retrofractamide A	-5.2	-0.22	153.36	-7.59	-7.54	-0.05	-0.62	2.39	-0.62	0.0	37.8
	Piperlongumine	-6.57	-0.29	15.25	-8.06	-8.02	-0.04	-0.83	1.49	-0.83	0.0	7.31
	Piperlonguminine	-7.33	-0.37	4.21	-8.33	-8.66	-0.17	-0.47	1.49	-0.47	0.0	6.15
GKRP	Piperine	-6.94	-0.33	8.18	-7.84	-7.83	-0.01	-0.44	0.89	-0.44	0.0	47.21
	Retrofractamide A	-7.78	-0.32	1.97	-10.17	-9.97	-0.19	-0.97	2.39	-0.97	0.0	43.07
	Piperlongumine	-6.05	-0.26	36.69	-7.54	-7.37	-0.17	-0.83	1.49	-0.83	0.0	52.19
	Piperlonguminine	-6.29	-0.31	24.62	-7.78	-7.63	-0.15	-0.47	1.49	-0.47	0.0	51.11
11β-HSD1	Piperine	-7.08	-0.34	6.51	-7.97	-7.86	-0.11	-0.48	0.89	-0.48	0.0	77.13
	Retrofractamide A	-6.4	-0.27	20.37	-8.79	-8.7	-0.09	-0.78	2.39	-0.78	0.0	84.46
	Piperlongumine	-6.21	-0.27	28.02	-7.7	-7.79	0.09	-0.88	1.49	-0.88	0.0	56.09
	Piperlonguminine	-6.27	-0.31	25.49	-7.76	-7.61	-0.15	-0.44	1.49	-0.44	0.0	79.97
GFPT	Piperine	-6.86	-0.33	9.43	-7.75	-7.52	-0.23	-0.5	0.89	-0.5	0.0	88.48
	Retrofractamide A	-4.7	-0.2	361.58	-7.08	-7.13	0.04	-0.73	2.39	-0.73	0.0	45.2
	Piperlongumine	-6.2	-0.27	28.57	-7.69	-7.6	-0.09	-0.51	1.49	-0.51	0.0	39.98
	Piperlonguminine	-6.29	-0.31	24.62	-7.78	-7.63	-0.15	-0.47	1.49	-0.47	0.0	51.11
PTB1B	Piperine	-6.8	-0.32	10.45	-7.69	-0.7.65	-0.04	-0.49	0.89	-0.49	0.0	61.41
	Retrofractamide A	-6.12	-0.26	32.76	-8.5	-8.43	-0.07	-0.78	2.39	-0.78	0.0	25.33
	Piperlongumine	-6.28	-0.27	24.84	-7.77	-7.91	0.14	-0.92	1.49	-0.92	0.0	63.28
	Piperlonguminine	-5.9	-0.3	46.99	-7.4	-7.29	-0.11	-0.43	1.49	-0.43	0.0	55.98

The qualities were registered with AutoDock tools while creating the compliances alongside their binding energies and other computational qualities and values. DPP-4: Dipeptidyl peptidase inhibitor 4; GKRP: Glucokinase regulatory protein; 11β-HSD1: 11β-Hydroxysteroid dehydrogenase type 1; GFPT1: Glutamine-fructose-6-phosphate transaminase 1; PTB1B: Protein tyrosine phosphatase 1B

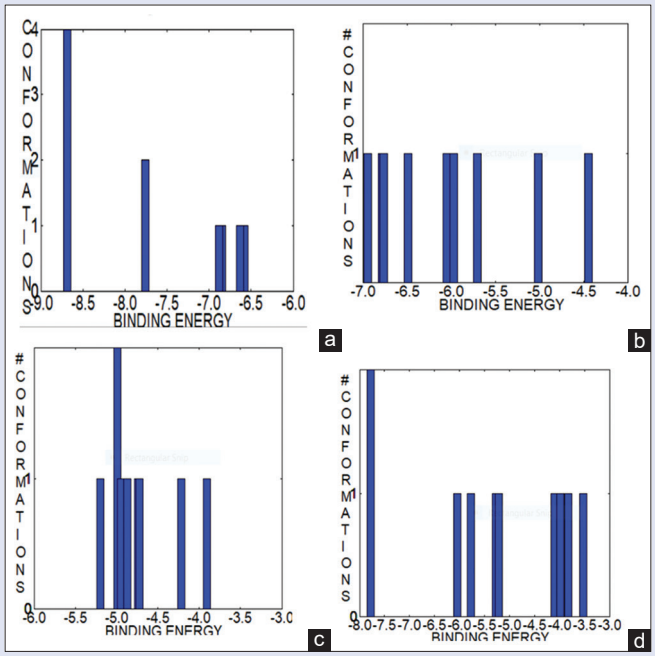

Figure 2: Graph depicting the binding energy versus conformations (2.0 clustering) of (a) piperine and dipeptidyl peptidase-4, (b) piperine and GKRP, (c) retrofractamide A and dipeptidyl peptidase-4, and (d) retrofractamide A and GKRP

point, and subsequently, it requests an *in silico* way to deal with its restorative possibilities.

The subatomic docking approach accurately foresees and recognizes the greatest restricting method of a given ligand in the dynamic site or restricting pocket of a given protein and effectively rank a group of ligands in agreement to their relating tentatively decided restricting affinities.[32] The pharmacophore examination clarifies the consequences of subatomic docking consequently further approving the coupling affinities along these lines supporting the *in silico* approach in building up of antidiabetic movement of bioactive compounds or ligands and foreseeing their job in bringing down hyperglycemia. Piperine and retrofractamide A demonstrated the least hindrance consistent 0.429 and 1.97 μM against DPP-4 and GKRP individually. This demonstrates their hindrance capacity to these receptors. *In vivo* examinations demonstrated that if both of these receptors can be repressed, high blood glucose levels can be limited. In this manner, the outcomes acquired in this examination obviously shows that restraint of receptors by the ligands piperine, piperlongumine, piperlonguminine, and retrofractamide A (acting as inhibitors) is upheld and further confirmed with the *in vivo* investigations,[33-38] which proposes that specific inhibitors can follow up on DPP-4, GKRP, 11β-HSD1, GFPT1, and PTB1B, which will encourage glucose digestion and increment insulin affectability. Individualistic approach for all the bioactive compounds mentioned here namely piperine, piperlongumine, piperlonguminine, and retrofractamide A can be further imposed and check for their more viable potentials against diabetes vehemently. If possible, then traditional and conventional approach for the use of these bioactive compounds in the plant shall be popularized not only in the rural areas but also in the suburban and urban areas too. *P. longum* is used in traditional medical practice in the Cook Islands wherein the leaves are pounded in a wooden bowl with little water and used to wash the chest of a person with suspected breast cancer. This application of *P. longum* to treat tumors is also recorded in Indian Ayurvedic medicine. *P. longum* is traditionally and internally

Table 5: Pharmacophore analysis showing the amino acids interactions of all bioactive compounds along with their position, chain, and the type of interactions

Receptor	Ligand	Amino acid(s) position(s) with chain	Type of interactions
DPP-4	Piperine	ALA564B , ILE529B	Hydrophobic
	Retrofractamide A	GLU408B, LEU366B, LEU410B	Hydrogen bond donor, hydrophobic
	Piperlongumine	THR565B, ILE529B, ALA564B	Hydrophobic
	Piperlonguminine	LEU504B, PHE559B, LEU477B, MET509B, ALA564B, THR565B, ILE529B	Hydrophobic
GKRP	Piperine	ALA520A , MET521A	Hydrophobic
	Retrofractamide A	MET521A, ALA520A, GLU31A, VAL9A, ILE10A	Hydrogen bond donor, hydrophobic
	Piperlongumine	ARG226A, ASN215A, ILE10A, LEU519A, ALA520A, VAL9A, TYR23A	Hydrogen bond acceptor, hydrophobic
	Piperlonguminine	MET212A, ALA26A, TYR23A, ARG226A, ALA520A, ILE10A, VAL9A	Hydrogen bond acceptor, hydrogen bond donor, hydrophobic
11β-HSD1	Piperine	ASN119C, GLY47C, ALA65C	Hydrophobic, hydrogen bond acceptor
	Retrofractamide A	LEU276A, MET233B, THR264B, LEU279A, TYR177B, ALA223B, ILE218B	Hydrophobic, hydrogen bond acceptor
	Piperlongumine	THR264C, LEU217C, ALA172C, VAL180C, TYR183C	Hydrophobic, hydrogen bond acceptor
	Piperlonguminine	MET233D, TYR177D, LEU171D, THR220D, ALA172D, ILE121D, ILE46D	Hydrophobic, hydrogen bond acceptor
GFPT1	Piperine	GLY587B, LEU571A	Hydrophobic, hydrogen bond acceptor
	Retrofractamide A	ILE588A, ALA574B, LEU575B, LEU571A	Hydrophobic
	Piperlongumine	LEU589B, TYR567B, LYS578A, GLY587B, LEU575B, LYS578B, ILE588A, ALA574B	Aromatic, hydrophobic, hydrogen bond acceptor
	Piperlonguminine	LEU575D, ILE588D, LEU571C	Hydrophobic
PTB1B	Piperine	ILE134A, PHE135A, GLU136A	Hydrophobic, hydrogen bond acceptor
	Retrofractamide A	HIS25A, ALA27A, ARG254A	Aromatic, hydrophobic, hydrogen bond acceptor
	Piperlongumine	GLU136A, ASP137A, GLY93A, ILE134A, MET133A	Hydrophobic, hydrogen bond acceptor
	Piperlonguminine	GLY93A, MET133A, PHE135A	Hydrophobic, hydrogen bond acceptor

DPP-4: Dipeptidyl peptidase inhibitor 4; GKRP: Glucokinase regulatory protein; 11β-HSD1: 11β-Hydroxysteroid dehydrogenase type 1; GFPT1: Glutamine-fructose-6-phosphate transaminase 1; PTB1B: Protein tyrosine phosphatase 1B

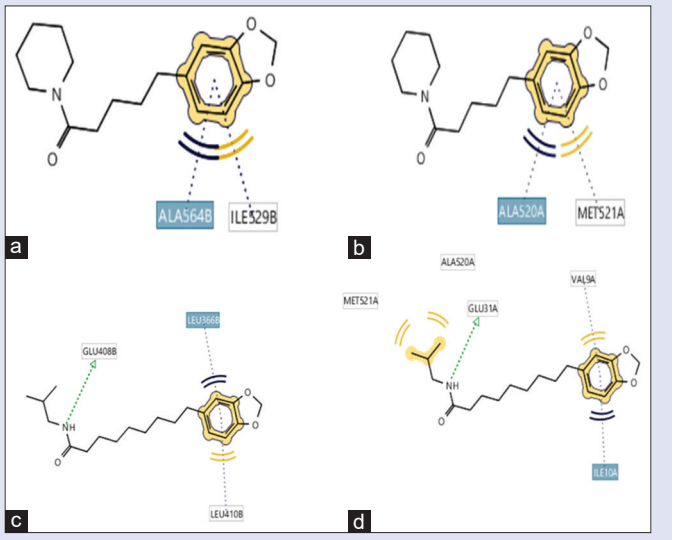

Figure 3: The pharmacophore analysis and amino acid positions of (a) piperine and dipeptidyl peptidase-4, (b) piperine and GKRP, (c) retrofractamide A and dipeptidyl peptidase-4, and (d) retrofractamide A and GKRP. The green dotted lines denote the hydrogen bond (donor); the yellow and the blue arcs denote the hydrophobic interactions among them

used in Chinese medicine to treat stomach chills, vomiting, etc., while in Ayurvedic medicine, it is used to treat colds, asthma, bronchitis, etc.

CONCLUSION

All the four ligands piperine, retrofractamide A, piperlongumine, and piperlonguminine indicated great subatomic docking results which were additionally confirmed with the pharmacophore investigation. Piperine with −8.69 kcal/mol restricting vitality and 0.429 μM hindrance consistent demonstrated the greatest potential to be a decent inhibitor against focused receptors. Even though the essential ideas of connection among ligands and focused on receptors had been as of now characterized, numerous inquiries still stayed indistinct for connection between docking result in AutoDock step and number of bonds in 2D structure of pharmacophore examination step. In this way, further research is required utilizing molecular dynamics simulations and hydrogen bond examination to unmistakably comprehend the strength of hydrogen bonds and hydrophobic connections between bioactive mixes and focused on receptors.

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Conflicts of interest

There are no conflicts of interest.

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