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In Silico Prediction of Antidiabetic Activity of Phytoconstituents of *Pterocarpus Marsupium* Targeting α-Amylase Enzyme

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Background Diabetes is characterized by a metabolic imbalance of blood sugar levels. a-amylase enzyme hydrolyzed starch into glucose units. Current therapy has significant side effects. Current investigation of in silico antidiabetic evaluation of phytoconstituents of Pterocarpus marsupium targeting a-amylase. Methods In silico studies were investigated to determine the binding affinity of phytoconstituents of Pterocarpus marsupium in additional with the crystal structure of a-amylase (PDB ID: 3BC9) with help of Pyrx in autodock vina software. Further, investigate the amino acid interaction residue and impacts on the inhibitory potential of the active phytoconstituents. Additionally, the pharmacokinetics and SwissADME and pkCSM were used as online servers for the toxic effects research. Further, studied the pocket region of amino acid for the binding of phytoconstituents using the Ramachandran plot. Result Molecular docking results proposed that pterostilbenes and liquirtigenin (-8.1 kcal/mol) had best docked against a-amylase as related to native ligand (-5.6 kcal/mol) and metformin (-5.3 kcal/mol). The active phytoconstituent has actively participated in interaction with the amino acid residue leads to blockage of a-amylase activity. Furthermore, the pharmacokinetic and In ADMET investigations, the phytoconstituents toxicological values are within allowable ranges. Conclusion The most promising outcome was revealed by the phytoconstituents of Pterocarpus marsupium that bind to a -amylase. However, it encourages the traditional practice of Pterocarpus marsupium and delivers vital information in drug development and clinical treatment. It promotes traditional approach of Pterocarpus marsupium and provides crucial knowledge for medical research and therapeutic care.

Keywords: ADMET; a-amylase; Diabetes; Docking; In silico; *Pterocarpus marsupium*.

Diabetes is the relative issue of the metabolism of starch, fats, and protein that deflect fasting and postprandial glucose level in the body. Diabetes is a chronic disease that impairs the metabolic regulation of blood sugar in the body. á-amylase enzyme hydrolyzed starch into glucose units. In diabetes, the frequency of non-insulin dependent is higher than that of insulin

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dependent.[1] Diabetes mellitus (DM) is a damaged retina, nephron, and neurons that leads to vascular difficulties at all ages people around the world. Currently, WHO has reported that more than 347 million people have worldwide been affected by diabetes, which will be the seventh-driving reason cause of death. In India, approximately 77 million individuals suffered from prediabetic stage.[2] However, the diabetic condition relative or all-out shortfall of insulin secreted from pancreas â-cell leads to elevated glucose in the body by insulin-sensitive tissues. Insulin has involved in the metabolisms of kinds of macromolecules like fats, proteins, and carbohydrates.[18] Thus, the deflection in the secretion or release may hamper all related functions. Therefore, the free unsaturated fats and glycerol levels rise in the blood. Furthermore, insulin-subordinate diabetes/ type-I is related to obliteration of â-cell while non-insulin subordinate/ type-II is obscure in the etiology and relates with a habitual and environmental problem. [3]

Pterocarpus marsupium is an herbal tree belonging Fabaceae family which is regularly found in the southern region of India and Srilanka. This tree is well-known as Malabar kino, Indian kino, Vijasar, Bijasar, and is a significant restorative plant. Hence, Charaka Samhita, which is acceptable to be one of the most seasoned and endured old compositions of Ayurveda concludes the *Pterocarpus marsupium* for treating diabetes mellitus.

Pterocarpus marsupium is propagated as a medium to an enormous tree with a stature of up to 30 meters. The outer bark is harsh and vertically cracked. The internal part of the heartwood is a brilliant yellowish shade while the outer sapwood is light yellow. The tree has *compound leaves*, made up of three leaflets and a spectacular golden *yellow blossom*.[4] The phytochemicals present in the *Pterocarcus marsupium* are pterostilbene, (-)-epicatechin, pterosupin, marsupsin, protein, pentosan, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, propterol B, marsupinol, irisolidone-7- O-A-L-rhamnopyranoside, alkaloids 0.4%, tannins 5%, Phydroxybenzaldehyde, beudesmol, erythrodirol-3-monoacetate, marsupol, carpusin, propterol have been obtained mainly from the heartwood and root. Multiple phytoconstituents found in *Pterocarpus marsupium's* diverse sections, which exhibit a wide variety of medicinal efficacy that contain antibiotics, antidiabetic, astringent, anti-diarrheal, and anti-hemorrhagic properties. Also, leaves are employed in the treatment of rheumatoid arthritis, skin diseases, fractures, ophthalmology, leprosy, rectalgia, and leucoderma, sores, constipation, hemorrhages skin diseases, depurative, boils, stomach pain, and gastrointestinal disorders. [5]

Molecular docking is the virtual screening computerized program for the prediction of a novel compound that can potentially for the treatment of a complex problem. It is a useful and novel technique to determine remedial dynamic mixtures at atomic level information of ligand-receptor binding. It is précised, fast, cost-effective, and time-saving techniques which eliminate the danger of animal trials.[6] However, there are few studies of phytoconstituents of *Pterocarpus marsupium* inhibit á-amylase. Therefore, in this present study we investigated the novel phytoconstituent of *Pterocarpus marsupium* against type-II diabetes targeted á-amylase enzyme by *in silico* method.[16]

MATERIAL AND METHODS

Molecular docking platform

Pyrx in autodock vina software was used to conduct a molecular docking analysis on all phytoconstituents of *Pterocarpus marsupium* that were chosen as ligands against the target á-amylase.[7, 17]

Selection of protein and preparation of its structure

Phytoconstituents were analysed by in silico method using the crystal structure of Alpha-amylase (PDB ID: 3BC9) complex with inhibitor 6-Amino-4-hydroxymethyl-cyclohex-4-ene-1,2,3-triol (Native ligand) involved in type II diabetes were downloaded in PDB format from the RCSB protein data bank (www.rcsb.org) R-value free was 0.178 and R-value work was 0.151 with resolution 1.35 selected for the present study. The structure of the protein target was prepared and refined using discovery studio visualizer 2021 (https://discover.3ds.com/) then use LigPlot+ v.2.2 to examine how ligands interact with amino acid residue.[8] (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) 3BC9 is a complex structure containing chains A, B, C, D, and E whereas chain A was used to prepare macromolecules and other

co-crystallized water molecules and non-standard residue, were removed and added the polar hydrogen atom. Energy minimization and addition of missing amino acid residue done using Swiss-Pdb viewer (https://spdbv.vital-it.ch/). To produce a protonation state at physiological pH, one uses autodock vina, build up geometry optimization, additional polar hydrogen, gasteiger charges, and Kollman charges.[9]

Selection of ligands and preparation of its structure

The three-dimensional structure of all phytoconstituents was retrieved from the PubChem database available on the NCBI website (https://pubchem.ncbi.nlm.nih.gov/). Energy minimization, geometrical confirmation, and hydrogen bond are supplemented done by the PyRx-virtual screening tool. All ligands were put into the PyRx virtual screening programme using the Open Babel control. SDF ligand files are converted to PDB format via the Open Babel programme.[10] Additionally, to obtain atomic coordinates for molecules, the Autodock Vina tool (http://vina.scripps.edu/) assists in identifying the torsion root, correcting torsion angles, altering charges, and universal force field optimization (UFF). All chemical structures are shown in Figure 1.

Receptor grid preparation

In order to anticipate biological activity, the fundamental objective of molecular docking was to assess the binding affinity and interaction between macromolecules and ligands based on geometry. PyRx was used for docking in this study's autodock vina virtual screening programme. For docking, the ligand and protein molecules are chosen. A mesh appears at the top of the protein structure. The size of the grid will be adjusted according to the binding pocket of the receptor at coordinate X, Y, and Z were set around the centroid of the active site to center X= 29.1394, Y= -1.5685, Z= 14.5186 and dimension coordinates at X= 89.5549, Y= 72.9128, Z= 69.6540. Further, PyRx in Autodock Vina will start. However, the protein-ligand interaction was analyzed using digital studio visualizer (DSV) 2021 (https://discover.3ds.com/) and LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/).

Molecular properties and ligand based ADME/T analysis

The pharmacokinetic study and toxicological parameter of the compound are important for the selection of potential drug candidates. We are selected the pkCSM and SwissADME online server tool for evaluation of molecular properties and toxicity study. The chosen ligands were further subjected to Lipinski rule of five screening using the criteria of molecular weight 500, logP 5, hydrogen bond acceptor 10, and topological polar surface area 140 (ú).[11]

PkCSM (http://biosig.unimelb.edu.au/pkcsm /prediction) is a database server online where ligands are evaluated for forecasting ADME and toxicological qualities by uploading SMILES of ligands. Additionally, the molecular characteristics such as molecular weight, logP, hydrogen bond donor, hydrogen bond acceptor, and topological polar surface area were obtained. Additionally, toxicological characteristics such as LD50, LOAEL, human maximum tolerated dose, AMES toxicity, hepatotoxicity, skin toxicity, T. pyriformis toxicity, hERG-I inhibitor, hERG-II inhibitor, and minnow toxicity were also obtained.[12]

SwissADME (http://www.swissadme.ch/index.php) is an online server in which ligands as uploaded Simplified Molecular Input Line Entry System notation (SMILES), followed by predicting various physicochemical and pharmacokinetic parameters of the ligands.[13]

Ramachandran Plot

The plot indicates the allowed region of á-amylase enzyme for the binding of phytoconstituents. It is determined by using discovery studio visualizer 2021.

Hydrophobicity Plot

The plot quantitatively determines the tendency of hydrophobicity or hydrophobicity of amino acids of enzyme (á-amylase). The plot is depicted from discovery studio visualizer 2021.

RESULT AND DISCUSSION

The alpha (á)-amylase is a metalloenzyme that includes calcium ion which helps in digestion with the cleavage of complex molecule polysaccharide into simpler form likewise glucose and maltose. Further, this enzyme leads to causes of hyperglycemia and elevated blood sugar level. á-Amylase is thrust area to therapeutic target for management postprandial elevated blood sugar level.[14] Currently, allopathic medications are

utilized to treat diabetic mellitus; nevertheless, these medications have had major negative side effects and disrupted people's regular lives. These days, herbal therapies are receiving greater attention for treating a variety of disorders since they have few side effects.[15] The objective of the current investigation was to assess the ability of plant phytoconstituents of *Pterocarpus marsupium*

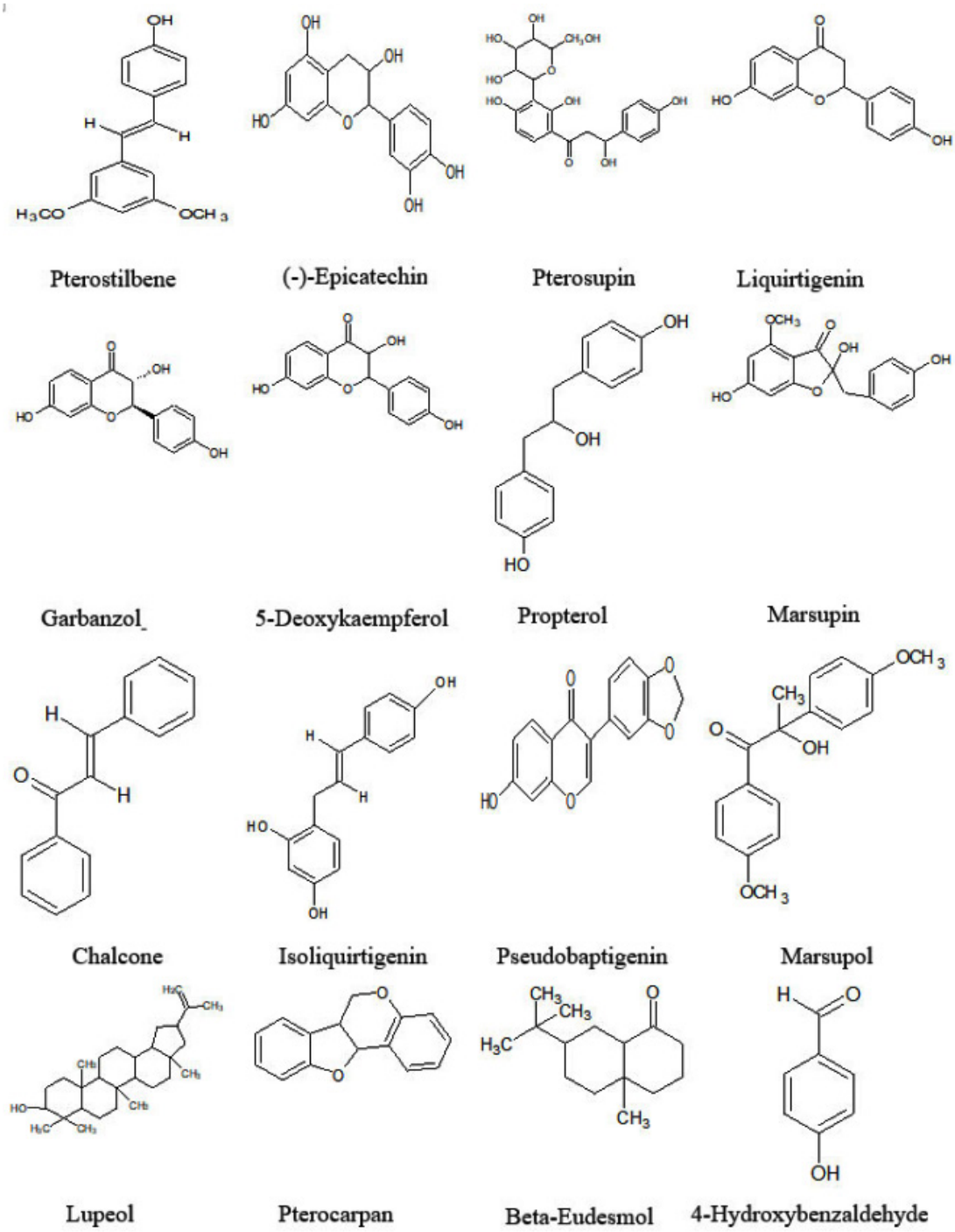


Fig. 1. Phytoconstituents of *Pterocarpus marsupium*

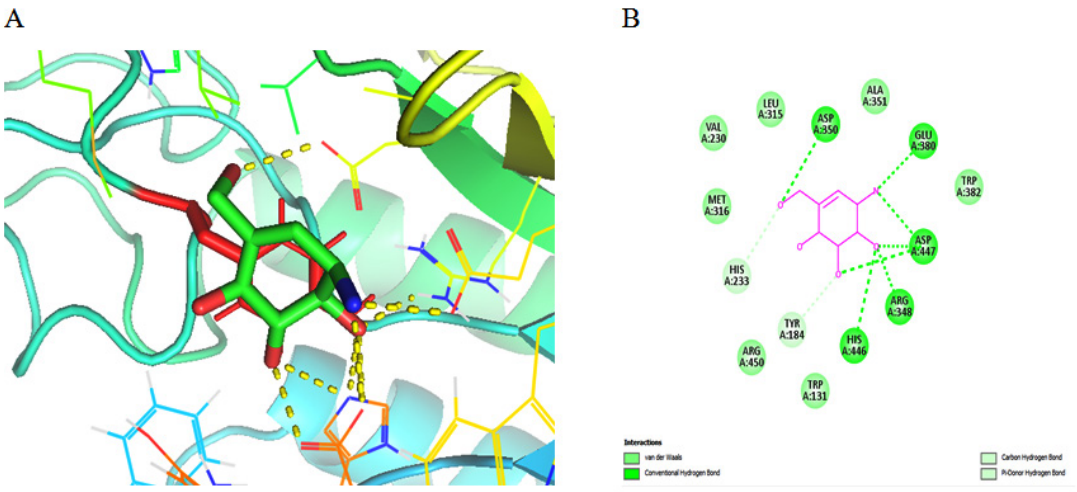
to inhibit the enzyme -amylase. In this work, we used Pyrx in autodock vina to do molecular docking experiments on all phytochemicals discovered in *Pterocarpus marsupium*, then we examined how these interactions with amino acid residues affected the activity of the active ingredients. Using the SwissADME and pkCSM online servers, selected ligands with the greatest match were further assessed for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics.

Molecular docking studies

Native ligand is present in the protein structure (PDB ID: 3BC9) with the co-crystallized form which re-docked exhibited the similar pattern of interaction as found in the reported in the

Table 1. Docking score of ligands against á-amylase (PDB ID: 3BC9)

Sr. No.	Ligands/Inhibitors	PubChem ID	Docking Score
1	Pterostilbene	5281727	-8.1
2	(-)-Epicatechin	72276	-7.9
3	Pterosupin	133775	-7.9
4	Liquirtigenin	114829	-8.1
5	Garbanzol	442410	-7.6
6	5-Deoxykaempferol	5281611	-7.1
7	Propterol	185124	-7.2
8	Marsupin	134369	-7.6
9	Chalcone	637760	-7.1
10	Isoliquirtigenin	638278	-7.6
11	Pseudobaptigenin	5281805	-8.1
12	Marsupol	349350392	-6.9
13	Lupeol	259846	-7.5
14	Pterocarpan	6451349	-7.4
15	Beta-Eudesmol	91457	-7.0
16	4-Hydroxybenzaldehyde	126	-5.4
17	Native ligand	193758	-5.6
18	Metformin	4091	-5.3
19	Voglibose	444020	-6.0



A. Re-docked native ligand (Green) whereas co-crystalized ligand (Red) at active site of protein using Pymol
B. Amino acid interaction visualization of re-docked ligands

Fig. 2. Overlapping structure of co-crystallized ligand and re-docked ligand

literature, along with binding energy -5.6 kcal/mol, where ASP350, GLU380, ASP447, ARG348, and ARG348 indicated hydrogen bonding catalytic residue and hydrophobic pocket region HIS233, ALA351, LEU315, VAL230, MET316, ARG450, TYR180, TRP131 and TRP382 (Figure 2).

The docking score and binding energy of all phytoconstituents of *Pterocarpus marsupium*

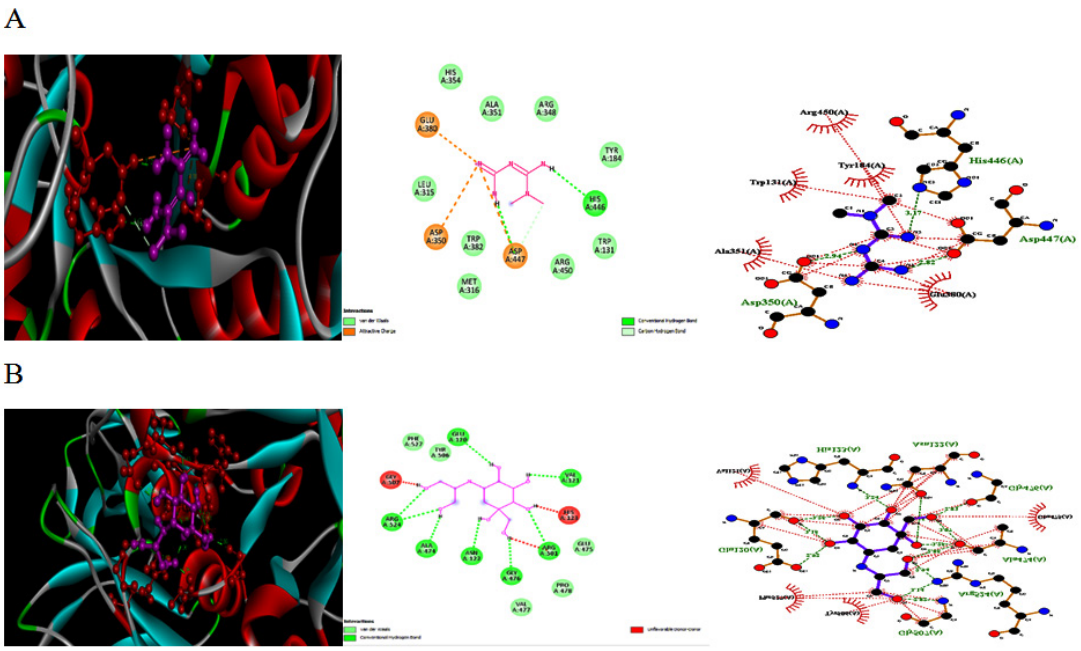


Fig. 3. Binding interaction and docked pose of Metformin (A) & Voglibose (B) targeting á-amylase (PDB ID: 3BC9). The ligand (Pink) and amino acid residue (Red) binding pocket represent in ball & stick

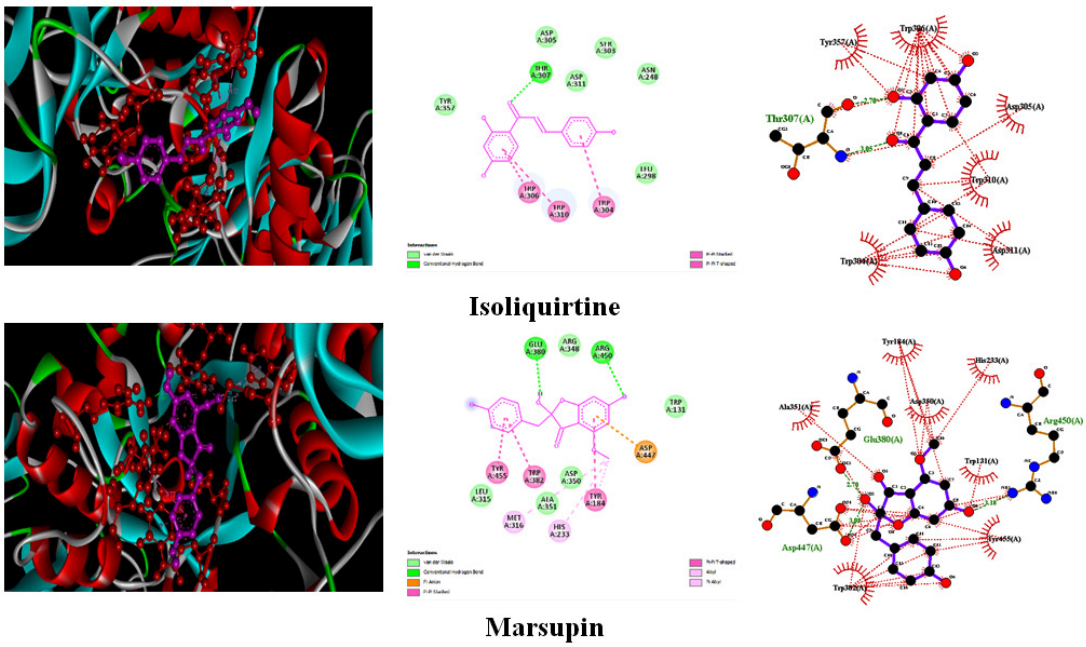


Fig. 4. Phytoconstituents binding interaction with á-amylase (PDB ID: 3BC9) visualized by DSV and LigPlot+ v.2.2

Table 2. Inhibitors interactions with the ±-amylase binding (PDB ID: 3BC9)

Ligands/ Inhibitors	Binding Energy (kcal/mol)	Amino acid interaction With hydrogen bond	Interaction distance (A)	With hydrophobic bond
Pterostilbene	-8.1	ASP350	2.75	HIS446, TYR184, TRP382, LYS353, ALA351, TYR455, MET316, ASP447
		HIS354	2.93	
Liquirtigenin	-8.1	ASP311	2.34	TRP310, TRP306, TRP304, TYR357, GLU312, ASN309, ASP305, LEU298,
		THR307	2.45	ASN248, SER303
Pseudobaptigenin	-8.1	No interaction		LEU315, MET316, HIS233, ASP447, TRP131, TRP382
(-)-Epicatechin	-7.9	THR307	2.58	ASP311, TRP310, TRP386, TRP304
		ASN309	2.6	
Pterosupin	-7.9	GLU586	2.81	MET490, SER461, ILE459, ASP484, TYR460, THR448, VAL457, ASP451
		TRP488	2.31	
		ASP449	2.48	
		SER458	2.34	
		ARG450	3.06	
Garbanzol	-7.6	ASP350	2.13	ARG450, GLU380, ALA351, TYR184, TRP131, TYR455, MET316, LEU315, HIS354, LYS353, TRP382, ARG348, ASP447
Marsupin	-7.6	GLU380	2.21	TYR455, TRP382, MET316, HIS233, TYR184, ASP447
		ARG450	2.35	
Isoliquirtigenin	-7.6	THR307	2.34	TRP306, TRP310, TRP304, TYR357, LEU298, ASP305, ASP311, SER303, ASN248
Lupeol	-7.5	ASP451	2.34	TRP488, TYR460, ILE459, LYS463, MET490, SER458, ASP449, ARG450
Pterocarpan	-7.4	No interaction		ASP447, ALA351, TRP382, TYR455, GLU380, LEU315, ASP350, ARG348
Propterol	-7.2	GLU380	2.19	ALA351, HIS354, TYR184, MET316, TRP382, LYS353, LEU 315, ARG450,
		ARG348	2.47	ASP350, TRP131, MET235, HIS446
		ASP447	2.22	
5-Deoxykaempferol	-7.1	No interaction		LEU315, MET316, HIS233, ASP447, TRP131, TRP382
Chalcone	-7.1	No interaction		TRP382, ASP447, TYR184, LEU315, GLU380, ALA351, ASP350, ARG346, TYR455, ARG450, TRP131, HIS446
Beta-Eudesmol	-7	TRP382	2.8	TYR455, ASP447, LEU315, GLU380, ALA351, HIS354
		LYS353	2.75	
Marsupol	-6.9	No interaction		HIS315, HIS354, ALA351, TRP131, ASP350, ARG348, LYS353
4-Hydroxybenzaldehyde	-5.4	TRP306	2.77	TRP310, ASP311, ASP313, GLU312
		THR307	2.25	

		THR307	2.77	
		ASN309	2.87	
Native ligand	-5.6	ASP350	2.72	HIS233, ALA351, LEU315, VAL230, MET316, ARG450, TYR180,
		GLU380	2.91	TRP131, TRP382
		ASP447	2.39	
		ARG348	2.61	
		ARG348	3.14	
		HIS446	2.78	
Metformin	-5.3	HIS446	2.42	GLU380, ASP350, ASP447, HIS446, LEU315, TRP382, MET316, ARG450,
		ASP447	2.12	TRP131, TYR184, ARG348, ALA351, HIS354
Voglibose	-6	GLU120	2.04	PHE527, TYR506, ARG501, HIS123, GLU475, PRO478, VAL477
		ARG524	2.65	
		ARG524	2.26	
		ALA474	2.56	
		ASN122	2.36	
		GLY476	2.87	
		ARG501	2.43	
		VAL121	3.1	

(-)-Epicatechin shows binding energy of 7.9 kcal/mol. it possesses a hydroxyl group which makes hydrogen bonding with amino acid THR307, ASN309 along with bond lengths 2.58 & 2.60. The presence of ketonic groups interacted with hydrophobic bonding *viz.* pi-stacking, pi-pi sigma with amino acid ASP311, TRP310, TRP386, TRP304. Pterosupin have containing hydroxyl group & ketone cyclic function shows binding energy -7.9 kcal/mol which is more than the reference drug. It possesses hydrogen interaction with amino acid residues GLU586, TRP488, ASP449, SER458, ARG450 at bond lengths 2.81, 2.31 2.48, 2.34 & 3.06 respectively. Also, Pterosupin has interacted with amino acid residue MET490, SER461, ILE459, ASP484, TYR460, THR448, VAL457, and ASP451 along with the pi-alkyl bond. Garbanzol has a binding energy of 7.6 kcal/mol with the interaction amino acid residues ASP350 2.13 ARG450, GLU380, ALA351, TYR184, TRP131, TYR455, MET316, LEU315, HIS354, LYS353, TRP382, ARG348, and ASP447. Marsupin has docked with binding energy 7.6 kcal/mol, it has hydroxyl groups involved in hydrogen bonding with amino acid residues GLU380, ARG450 with bond lengths 2.21 & 2.35. TYR455, TRP382, MET316, HIS233, TYR184, ASP447.

Table 3. ADME and toxicity profiles of ligands with high docking scores

ADMET Properties	Molecular formula	Molecular weight [g/mol]	Log P	TPSA [A^0]	HB Donor	HB Acceptor	Aqueous solubility [log mol/ L]	Human intestinal absorption (%)	Blood brain barrier
Pterostilbene	C16H16O3	256.30	3.02	38.69	1	3	-3.905	92.395	0.317
(-)-Epicatechin	C15H14O6	290.27	1.47	110.38	5	6	-3.117	68.829	-1.054
Pterosupin	C21H24O10	436.41	2.35	188.14	8	10	-2.791	49.815	-1.256
Liquirtigenin	C15H12O4	256.25	1.73	66.76	2	4	-3.304	94.333	0.375
Garbanzol	C15H12O5	272.256	1.7751	86.99	3	5	-2.788	93.102	-0.586
5-Deoxykaempferol	C15H10O5	270.24	1.73	90.90	3	5	-3.405	92.943	-0.864
Propterol	C15H16O3	244.29	1.95	60.69	3	3	-3.063	91.686	-0.087
Marsupsin	C16H14O6	302.28	1.20	96.22	3	6	-3.126	74.906	-0.794
Chalcone	C15H12O	208.26	2.54	17.07	0	1	-4.461	94.977	0.56
Isoliquirtigenin	C15H12O4	256.25	2.02	77.76	3	4	-3.06	91.096	-0.717
Pseudobaptigenin	C16H10O5	282.25	2.48	68.90	1	5	-3.084	97.239	-0.309
Lupeol	C30H50O	426.72	4.68	20.23	1	1	-5.861	95.782	0.726
Pterocarpan	C15H12O2	224.25	2.51	18.46	0	2	-3.86	99.271	0.315
Beta-Eudesmol	C15H26O	222.37	3.06	20.23	1	1	-4.9	94.296	0.634
4-Hydroxy benzaldehyde	C7H6O2	122.12	0.99	37.30	1	2	-0.985	87.261	-0.217
Metformin	C4H11N5	129.16	0.34	91.49	3	2	-2.707	59.401	-0.946
Voglibose	C10H21NO7	267.28	0.52	153.64	8	8	-1.724	24.211	-1.321

ADMET Properties	P-glycoprotein substrate	Total clearance [log ml/min.kg]	Bioavailability score	AMES Toxicity	Max. tolerated dose [log mg/kg.d]	hERG-I Inhibitors	hERG-II Inhibitors
Pterostilbene	YES	0.228	0.55	YES	0.438	NO	NO
(-)-Epicatechin	YES	0.183	0.55	NO	0.438	NO	NO
Pterosupin	YES	-0.004	0.55	NO	0.634	NO	NO
Liquirtigenin	YES	0.065	0.55	NO	-0.351	NO	NO
Garbanzol	YES	0.007	0.55	YES	0.147	NO	NO
5-Deoxykaempferol	YES	0.492	0.55	NO	0.479	NO	NO
Propterol	YES	0.081	0.55	NO	0.52	NO	NO
Marsupsin	YES	0.151	0.55	NO	0.048	NO	NO
Chalcone	YES	0.223	0.55	NO	1.031	NO	NO
Isoliquirtigenin	YES	0.087	0.55	NO	0.118	NO	NO
Pseudobaptigenin	NO	0.153	0.55	YES	0.116	NO	YES
Lupeol	NO	0.153	0.55	NO	-0.502	NO	YES
Pterocarpan	YES	0.107	0.55	YES	0.285	NO	NO
Beta-Eudesmol	NO	1.032	0.55	NO	-0.22	NO	NO
4-Hydroxybenzaldehyde	NO	0.565	0.55	NO	1.248	NO	NO
Metformin	YES	0.1	0.55	YES	0.902	NO	NO
Voglibose	YES	0.906	0.55	NO	1.453	NO	NO

ADMET Properties	Acute oral rat toxicity LD50 [mol/kg]	Oral rat chronic toxicity [log mg/kg bw/day]	Hepatot-oxicity	Skin sensitization	T.pyriformis Toxicity [log ìg/L]	Minnow toxicity [log/mmol/L]	Lippinskies rule violations
Pterostilbene	2.057	1.773	NO	NO	1.286	0.459	NO
(-)-Epicatechin	2.428	2.5	NO	NO	0.347	3.585	NO
Pterosupin	2.501	4.509	NO	NO	0.285	6.549	NO
Liquirtigenin	2.365	2.132	NO	NO	0.44	1.21	NO
Garbanzol	2.248	2.186	NO	NO	0.404	2.23	NO
5-Deoxykaempferol	2.428	1.977	NO	NO	0.474	1.536	NO
Propterol	2.456	1.577	NO	NO	0.76	1.555	NO
Marsupsin	1.918	2.702	NO	NO	0.368	1.854	NO
Chalcone	1.843	1.209	NO	YES	1.349	0.835	NO
Isoliquirtigenin	2.427	2.049	NO	NO	0.691	2.081	NO
Pseudobaptigenin	2.422	1.278	NO	NO	0.383	-0.344	NO
Lupeol	2.563	0.89	NO	NO	0.316	-1.696	NO
Pterocarpan	2.258	1.971	NO	NO	1.076	0.134	NO
Beta-Eudesmol	1.727	1.304	NO	YES	1.805	0.412	NO
4-Hydroxy benzaldehyde	1.902	2.635	NO	YES	-0.133	1.883	NO
Metformin	2.453	2.158	NO	YES	0.25	3.972	NO
Voglibose	1.989	4.454	NO	NO	0.285	6.767	YES

targeting á-amylase and interaction of amino acid residue with bonding distance are shown in Tables 1 & 2, respectively.

The docking binding energy of placed native ligand at 17th position and reference standard compounds [Metformin & Voglibose] at 18th & 19th position which is lower than many phytoconstituents of *Pterocarpus marsupium* .[20] The reference standard binding energy of metformin & voglibose is -5.3 kcal/mol & -6.0 kcal/mol, respectively. Metformin interacts with amino acid along with conventional hydrogen bonding HIS446 & ASP447, and hydrophobic interaction GLU380, ASP350, ASP447, HIS446, LEU315, TRP382, MET316, ARG450, TRP131, TYR184, ARG348, ALA351 & HIS354. Voglibose fit in protein pocket with hydrogen bonding is GLU120, ARG524, ARG524, ALA474, ASN122, GLY476, ARG501, and VAL121 and hydrophobic amino acid interaction with PHE527, TYR506, ARG501, HIS123, GLU475, PRO478, and VAL477 (Figure 3).

Analysis of binding affinity of selected phytoconstituents of *Pterocarpus marsupium* in the ranges of -8.1 to -5.4 kcal/mol. from docked result, it is observed that Pterostilbene, Liquirtigenin and Pseudobaptigenin exhibit highest binding affinity (-8.1 kcal/mol) in complex with á-amylase, as compared to other phytoconstituents *viz.* (-)-Epicatechin (-7.9 kcal/mol), Pterosupin(-7.9 kcal/mol), Garbanzol (-7.6 kcal/mol), 5-Deoxykaempferol (-7.1 kcal/mol), Propterol (-7.2 kcal/mol), Marsupin (-7.6 kcal/mol), Chalcone (-7.1 kcal/mol), Isoliquirtigenin (-7.6 kcal/mol), Marsupol (-6.9 kcal/mol), Lupeol (-7.5 kcal/mol), Pterocarpan (-7.4 kcal/mol), Beta-Eudesmol (-7.04 kcal/mol) and 4-Hydroxybenzaldehyde (-5.4 kcal/mol), respectively.

Based on visual inspection, computational docking of phytocompounds on á-amylase

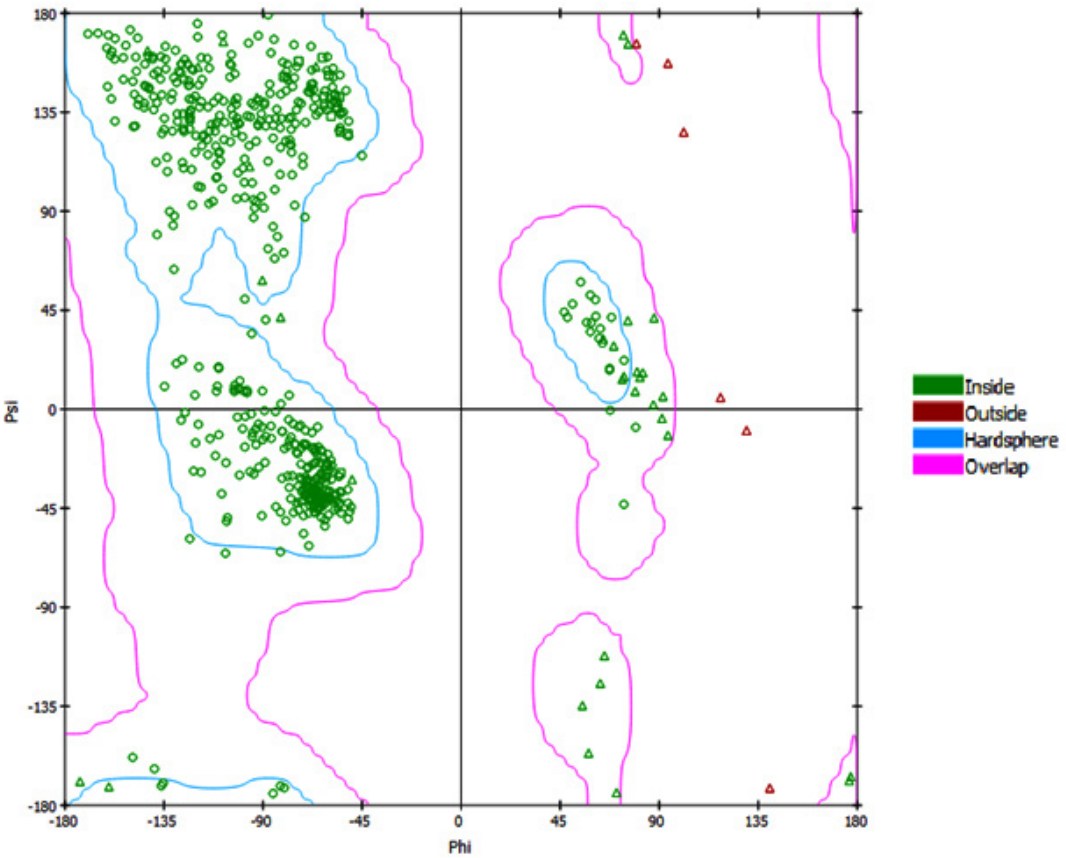


Fig. 5. Ramachandran plot showing allowed region in á-Amylase

substantially involves many types of interactions, including hydrogen bonds and hydrophobic bonds, alkyl, pi-stacking, and pi-alkyl interaction for the stable complex with -amylase. Additionally, it showed that voglibose and metformin had a similar binding pattern to á-amylase.

Pterostilbene, Liquirtigenin, and Pseudobaptigenin were suitably positioned in the pocket of á-amylase based on the docking results. The hydrogen interaction between the hydroxyl group of pterostilbene and the ASP350 and HIS354 molecules, with bond lengths of 2.75 and 2.93, is attributed to the formation of the -alkyl and -cationic & anionic interaction and the binding pocket formed by HIS446, TYR184, TRP382, LYS353, ALA351, TYR455, MET316, and ASP447 on -amylase (Figure 4). Pterostilbene is reported to have antidiabetic effect when fed orally to streptozotocin (STZ)-nicotinamide-induced diabetic rats for 2, 4, and 6 weeks.[11] Due to the presence of a C=O group and a centroid benzene ring that forms a hydrophobic pocket area with TRP310, TRP306, TRP304, TYR357, GLU312, ASN309, ASP305, LEU248 & SER303 on á-amylase, Liquirtigenin has created hydrogen bonding interactions with ASP311 & THR307 at 2.34 (ú) & 2.45 (ú). These observations are reported in the literature that liquirtigenin shows antidiabetic activity in streptozotocin induced Swiss albino mice by inhibiting á-amylase. Pseudobaptigenin is appropriately covered the hydrophobic pocket region of the protein with LEU315, MET316, HIS233, ASP447, TRP131 & TRP382. Vijayan et al, has reviewed *Pterocarpus marsupium* showed the anti-diabetic potential for the management of hyperglycemia, along with good antioxidant activity.

Isoliquirtigenin having binding energy -7.6 kcal/mol with the presence of hydroxyl function build conventional hydrogen bonding THR307 with bond length 2.34. Also, hydrophobic interaction with pi-pi stacking bond at amino acid residues TRP306, TRP310, TRP304, TYR357, LEU298, ASP305, ASP311, SER303, ASN248. Lupeol having binding energy -7.5 kcal/mol at the presence of hydroxy function stabilized conventional hydrogen bonding ASP451 with bond length 2.34 and also, interacted with amino acid residue pocket TRP488, TYR460, ILE459, LYS463, MET490, SER458, ASP449, ARG450. Pterocarpan has binding energy -7.4 kcal/mol with no hydrogen bond interaction. Also, binding pocket region amino acid residue ASP447, ALA351, TRP382, TYR455, GLU380, LEU315, ASP350, ARG348. Propterol having binding energy -7.2 kcal/mol with conventional hydrogen binding GLU380, ARG348, & ASP447 at bond length 2.19, 2.47, & 2.22 respectively. Also hydrophobic pocket region ALA351, HIS354, TYR184, MET316, TRP382, LYS353, LEU 315, ARG450, ASP350, TRP131, MET235, HIS446. 5-Deoxykaempferol having binding energy -7.1 kcal/mol with no

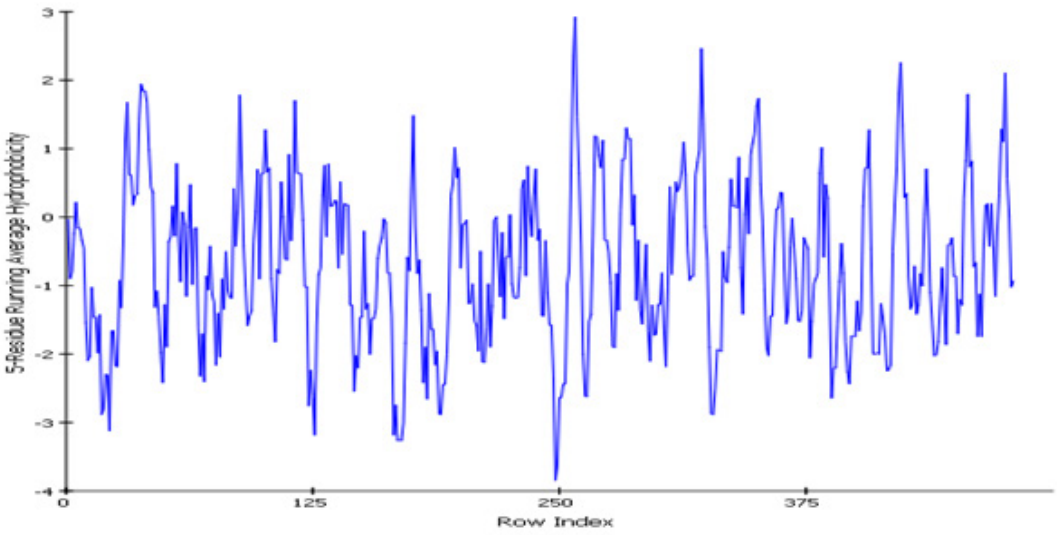


Fig. 6. Hydropathy plot showing tendency of phytoconstituent to fold in solvent (Hydrophobic).

hydrogen interaction still entrapped at pocket region LEU315, MET316, HIS233, ASP447, TRP131, TRP382. Chalcone is having binding energy -7.1 kcal/mol at none hydrogen bonding and also, fits pocket region TRP382, ASP447, TYR184, LEU315, GLU380, ALA351, ASP350, ARG346, TYR455, ARG450, TRP131, HIS446. Moreover, Beta-Eudesmol binds at binding energy -7.0 kcal/mol with conventional hydrogen binding TRP382, LYS353 at bond length 2.80, & 2.75. Also, pi-sigma and pi-alkyl interaction TYR455, ASP447, LEU315, GLU380, ALA351, HIS354. Marsupol is bound with binding energy -6.9 kcal/mol at no hydrogen interaction. Also, hydrophobic interacted amino residue pocket region HIS315, HIS354, ALA351, TRP131, ASP350, ARG348, and LYS353. 4-Hydroxybenzaldehyde is showing binding energy -5.4 kcal/mol with conventional hydrogen interaction TRP306, THR307, THR307, and ASN309 with bond lengths 2.77, 2.25, 2.77, and 2.87. Also, hydrophobic pocket region TRP310, ASP311, ASP313, GLU312. However, anti-hyperglycemic effect of *Pterocarpus marsupium* seed extract (100 mg/kg and 200 mg/kg) on gabapentin-induced hyperglycemia in Wistar albino rats was tested *in vivo.* Further, the study suggested that on clinical-based 52 patients had a trial for 12 weeks of *Pterocarpus marsupium* significantly using the student's paired t-test, medications that were demonstrated to reduce fasting blood glucose, postprandial blood glucose, and glycosylated haemoglobin were compared to baseline. It is statically significant by p-value calculated for all parameters is 0.05. However, the study supports our finding of potential lead moiety in *Pterocarpus marsupium* to achieve the antidiabetic effect.

ADME and toxicity studies

The pharmacokinetic and toxicological profile of phytoconstituents is critical for transforming a chemical into a powerful medicine. In this work, we use the SwissADME and pkCSM servers to conduct ADMET investigations. The partition coefficient (log P) and total polar surface area (TPSA) of the molecule define its absorption and lipophilicity potential, respectively. When the TPSA is greater than 140, the drug molecule easily penetrates the cell membrane. However, the optimal Log P value of pharmaceuticals is critical for the specific pharmacological target. The Log P-value for oral and sublingual absorption is 1.35 - 1.80; sublingual absorption is greater than 5, while the central nervous system is less than 2. Furthermore, drug penetration to the blood-brain barrier (BBB) ranges between -3.0 and 1.2, whereas ligand aqueous solubility ranges between -6.5 and 0.5. Additionally, P-glycoprotein substrate is responsible for the efflux of a substrate from inside to outside the cell, and its inhibition causes drug resistance. Further, human intestinal absorption (HIA, %) of ligands are characterized into low, medium and high value ranges 0 – 29 %, 30 – 79%, and 80 – 100 %, respectively.

Based on the ADMET studies, all the selected ligands obey Lipinski's rule. Followed, all ligands are an acceptable range for TPSA, Log P, and BBB parameters, and also, ligands are satisfied % HIA, bioavailability score, and total clearance. Furthermore, the ligands were expected to be free of AMES toxicity, hepatotoxicity, and skin sensitivity. Table 3 shows that it did not suppress hERG-I (low risk of cardiac toxicity), Tetrahymena pyriformis toxicity, minnow toxicity, maximum tolerated dosage, rat acute oral toxicity, and chronic toxicity. Erstwhile, in vivo evidence revealed that *Pterocarpus marsupium* seed ethanolic extract at doses of 100 mg/kg and 200 mg/kg had significantly lower blood glucose levels on days 1, 7, 14, and 21 compared to disease control rats. Furthermore, various extracts of *Pterocarpus marsupium* (100 mg/kg body weight) administered to streptozotocin induces diabetes rat with continuous 10 days reduced blood sugar level, and serum insulin level. Additionally, several phytoconstituents such flavonoids, terpenoids, cardiac glycosides, alkaloids, glycosides, saponins, tannins, proteins, and carbohydrates in ethanol, acetone, and isopropyl alcohol (IPA) (1:1) extracts of *Pterocarpus marsupium* stem wood.[19] This investigation supports our *in silico* study that pterostilbenes and liquirtigenin have the lowest binding energy (-8.1 kcal/mol) with complex á-amylase to reduce blood sugar levels. Hence, it is manifest that our study screened phytoconstituents have binding energy, docking score, and interaction with catalytic residue, leading to blocking the action of á-amylase to restrict diabetes. Amongst all phytoconstituents only pterostilbenes and

liquirtigenin show potential inhibition of á-amylase metalloenzyme to regulate the blood sugar in the body.

Ramachandran Plot

In the present study, á-amylase enzyme had the presence of the core region for the binding of ligands. The phi-psi torsion angles of all residues in the protein structure are depicted in Figure 5. The presence of green dots reflects the core region, which is the best phi-psi value which is indicted the percentage of residues to binding with ligands.

Hydrophobicity Plot

This plot is numerical representation of degree of hydrophobicity or hydrophilicity of the á-amylase enzyme. The x-axis shows amino acid sequence of protein, while y-axis represent degree of hydrophobicity or hydrophilicity shown in Figure 6. Our plot depicted that the á-amylase enzyme highly interacted with ligands.

CONCLUSION

Pterocarpus marsupium, a medicinal plant traditionally used as an antidiabetic, has phytoconstituents with the ability to cure diabetes. The results of a computationally guided algorithm screening of chosen phytoconstituents of the plant were highly selective, had great binding potential, and had the best match with á-amylase enzyme. It has been hypothesized that pterostilbene and liquirtigenin may have strong inhibitory effects against á-amylase based on the analysis of the ligands with the highest docking scores that are positioned at the site of inhibition, interaction profiles with catalytic residues, and acceptable ADMET parameters. The most encouraging outcome was shown by the phytoconstituents of *Pterocarpus marsupium* that were targeted á-amylase. However, it supports *Pterocarpus marsupium* customary behaviour and provides crucial data for *clinical* diagnosis and therapeutic research.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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