ORIGINAL ARTICLE

In silico elucidation of *Boesenbergia rotunda* phytoconstituents against diabetes mellitus

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Abstract

Introduction: Herbal medicine has become a well-chosen treatment to reduce the negative effects of diabetes mellitus (DM) and its serious complications due to its side effects and low cost. **Material and Methods:** Plants of the Zingiberaceae family, for example, *Boesenbergia rotunda, Renealmia alpinia,* and *Zingiber zerumbet* have been extensively investigated for their phytoconstituents and molecular mechanisms. This study aims to examine the molecular interactions that exist between the various bioactive chemicals in *B. rotunda* and that targeted proteins associated with type 2 DM. Molecular docking studies were done to assess the binding mode and interactions of synthesized hits at binding site of receptors. **Results:** Results of *in silico* studies showed that polyphenols and flavonoids have excellent drug-likeness properties, pharmacokinetic profile against DM targets such as peroxisome proliferator-activated receptor gamma (PPARG), dipeptidyl peptidase 4 (DPP4), and α-glucosidase. Molecular docking results highlighted five of top 10 interactions correspond to pinocembrin, alpinetin, and pinostrobin with DPP4, α-glucosidase, and PPARG; pinocembrin and silybin with PPARG. These proteins involved in regulating the functions such as inflammation, insulin resistance, oxidative stress, glucose, and lipid metabolism. **Conclusion:** This work provides a dynamic state of *B. rotunda*, especially flavonoids that show their diabetic benefits.

Key words: peroxisome proliferator-activated receptor gamma, diabetes mellitus, flavonoids, molecular docking, *Boesenbergia rotunda*

INTRODUCTION

Diabetes is a metabolic disorder characterized by disruption of carbohydrate, protein, and lipid metabolism and disorders such as microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart diseases and stroke). The International Federation of Diabetes estimates that 425 million people are diagnosed with type 2 diabetes mellitus (T2DM) worldwide.[3] T2DM is a complex disease characterized by high levels of plasmatic glucose. It includes various cellular mechanisms such as insulin secretion, insulin resistance, and carbohydrate absorption. Other proteins identified as playing key role in the formation of T2DM are glucokinase, AMP-activated protein kinase, 11β-hydroxysteroid dehydrogenase, insulin receptor substrate, interleukin 1 beta, dipeptidyl peptidase 4 (DPP4), glutamine fructose-6-phosphate, amidotransferase, peroxisome proliferator-activated gamma receptor, protein tyrosine phosphatases, tyrosine kinase insulin receptor, and protein kinase B.[4]

Fingerroot is a genus of ginger (Zingiberaceae). The annual crop, also native to the southern province of Yunnan, China, west of Malaysia, grows in the tropical rain forest. It has an underground stem, known as the rhizome.[1,2] These spread into many groups in the same way as bananas, ginger, galangal, and turmeric. *Boesenbergia rotunda* contains various phytoconstituents, divided into two main groups – namely, flavonoids and polyphenols (chalcone derivatives such as pinocembrin, pinostrombin, alpinetin, panduratin, cardamonin, quercetin, and kaempferol), which may indicate a significant benefit of drug availability. This plant acts as a versatile use of antiulceration, hepatoprotective, helicobacter pylori inhibitor, anti-inflammatory, anticancer, antiallergic,

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antibacterial, antioxidant, anti-dengue viral, antiherpes viral, wound healing, antimutagenic, antifungal, analgesic, antipyretic, antispasmodic, insecticidal, larvicidal, and pupicidal activities.

In addition, they promote insulin production and glucose homeostasis, but despite this information, little is explained about the processes and proteins targeted to regulate glucose levels, insulin resistance, insulin production, and inflammation.[5] The mechanisms of action that has been linked to diabetes are insulin resistance and carbohydrate absorption. The search for new therapeutic purposes remains a challenge, although there are many different flavonoid groups in the treatment of DM that has not been properly investigated. Taken together, the purpose of this work is to investigate the fingerroot flavonoids and confirms the strong diabetes properties and *in silico* method by predicting the binding interactions between flavonoids and proteins to identify antidiabetic activity.

The purpose of the study is to present a list of bioactive compounds of *B. rotunda* and determine how they interact with the essential proteins in type 2 DM [Figure 1].

MATERIALS AND METHODS

Construction of Phytochemical Library

In this study, first of all, text mining analysis of *B. rotunda* and its disease association was carried out using server Disease List Automatically Derived For You based on the highest score with diabetes. However, the compounds which prove the antihyperglycemic activity are unknown. Hence, to find the antidiabetic candidates from *B. rotunda,* a library of phytochemicals in sdf and mol2 format was constructed through searching scientific literature, PubChem, and ZINC database.[6]

Preparation of Protein Targets Against Type 2 Diabetes

To discover new potential antidiabetic candidates, we searched the molecular targets of T2D in Potential Drug Target Database (www.dddc.ac.cn/pdtd), DrugBank (www.drugbank.ca), pharmGkb (www.pharmgkb.org), and Therapeutic Targets Database (bidd.nus.edu.sg/group/ttd) which resulted in several molecular targets against T2D. The X-ray crystallographic structures of the protein targets were downloaded from the Protein Data Bank (www.pdb.org) and used as the receptors in the molecular docking, that is, DPP4, PID:2p8s; PPAR gamma, PID: 5ycp; Alpha glucosidase, PID: 5nn5; and PTP1B, PID: 1c83. The structures were edited to remove water, ligands, and heteroatoms (HETATM) using Discovery Studio Visualizer v 19.1.0 (BIOVIA, San Diego, CA, USA). Preparation of receptor files involves changing atom type, removing water molecules, adding polar hydrogen atoms, Gasteiger charges, and conversation into PDBQT format using AutoDockTools v1.5.6.

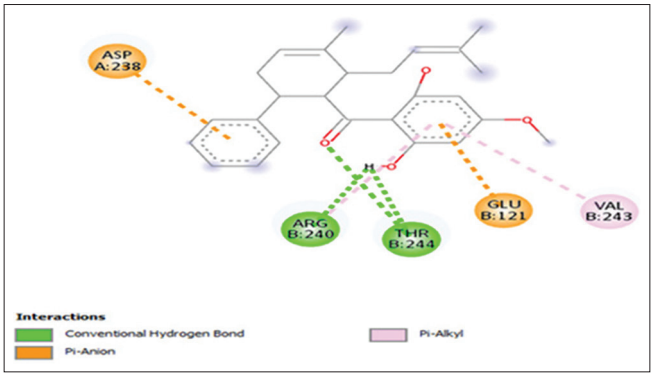

Figure 1: 2D interaction of pinocembrin with peroxisome proliferator-activated receptor gamma

Preparation of Ligands

From *B. rotunda,* polyphenols and flavonoids are selected for docking simulation such as pinocembrin, alpinetin, quercetin, silybin, panduratin A, boesenbergin, and pinostrombin. The sdf file of ligands is retrieved from PubChem.

Docking Simulation

Molecular docking studies were carried out for the selected molecules in the binding site of target proteins using AutoDock Vina and AutoDock tools.[7] The grid box size was set for each receptor and the exhaustiveness was set to 24. The results with the best conformation and energy were selected for further analysis. Discovery Studio Visualizer V19 was used for visualization and analysis of the protein-ligand complexes. Autodock Vina was employed for binding affinity measurement. The content of configure file was determined as position of receptor file, ligand file, data of Grid box's three coordinates X, Y, and Z were 18.125, −27.72, and −0.34, respectively, in case of 11β-HSD1, 8.82, 5.31, and −7.903 for GFAT, −11.21, −22.77, and −6.75 in PTP1B, 14.5, −18.02, and 17.04 in SIRT6, the size of Grid box which was set up in 30 × 30 × 30 points, number of modes which were 10, and the energy range which was set up at 9 kcal/mol. Docking process in AutoDock Vina has been performed with 1000 of exhaustiveness for enhancing accuracy [Figure 2].[8]

Pharmacophore Studies

Common pharmacophores for the ligands were analyzed using PharmaGist and ZINCPharmer web servers. The control inhibitors for all therapeutics targets were downloaded from PubChem. All screened phytochemicals were further used for ligand-based pharmacophore studies.[6]

Drug-Likeness Prediction

Molinspiration server was used to perform QSAR studies to find the suitability of screened phytochemicals as a drug

candidate. Drug-likeness based on Lipinski's rule, Ghose Filter, MDDR-like rules, and Veber rule was calculated using ADMET lab (http://admet.scbdd.com/calcpre/calc_rules/) and Molsoft (https://molsoft.com/mprop/). According to MDDR-like rules the drug-like molecules should have no. of rings ≥3; no. of rigid bonds >18, and no. of rotatable bonds >6. As per Veber rule, the drug-like molecules have rotatable bond count ≤ 10 and TPSA ≥140. According to Lipinski rule, Log P≤ 5, molecular weight ≤500, no. of hydrogen bond acceptors ≤10 and no. of hydrogen bond donors ≤5. These rules were applied to select probable ligands.[9,10]

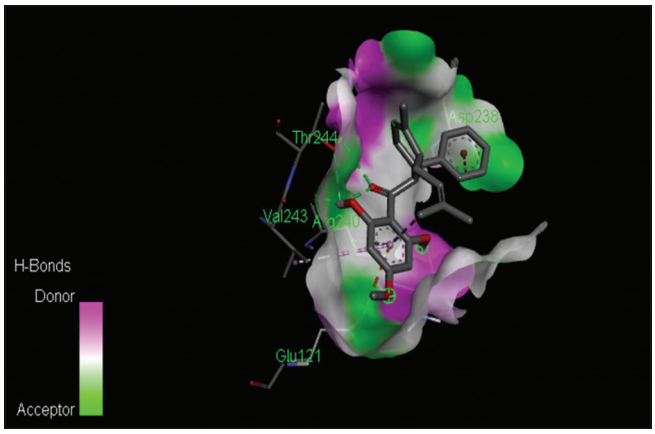

Figure 2: 3D interaction of pinocembrin with peroxisome proliferator-activated receptor gamma

ADME Prediction

The pharmacokinetic properties of the ligands were predicted using Swiss ADME (http://www.swissadme.ch/), ADMET lab. For predicting the toxicity behavior of the screened phytochemicals, we used admetSAR prediction tool (http/://lmmd.ecust.edu.cn:8000) [Figures 3 and 4].[11]

RESULTS

Molecular docking studies

Molecular docking results highlighted five of top 10 interactions correspond to pinocembrin, alpinetin, and pinostrobin with DPP4, α-glucosidase, and PPARG; pinocembrin and silybin with PPARG. These proteins involved in regulating the functions such as inflammation, insulin resistance, oxidative stress, glucose, and lipid metabolism.

DISCUSSION

Natural products have immense potential in the management of diabetes. T2D is one of the major health problems all over the world in all age groups and both the sexes. In spite of the presence

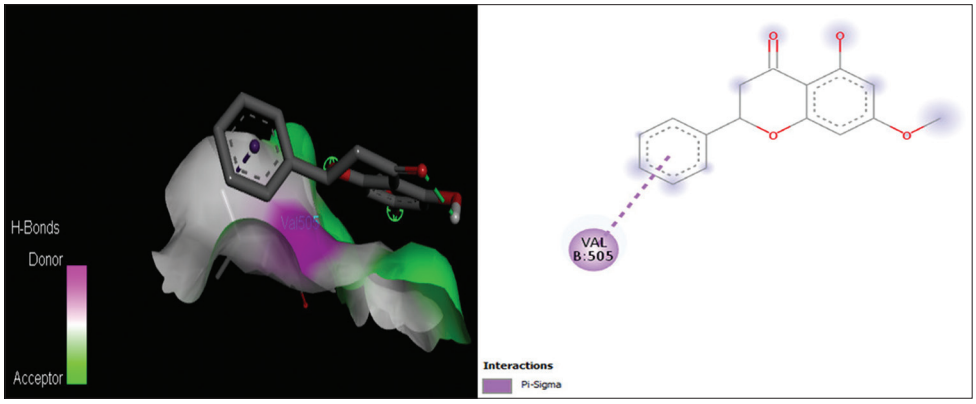

Figure 3: 3D and 2D interactions of pinostrombin with α-glucosidase

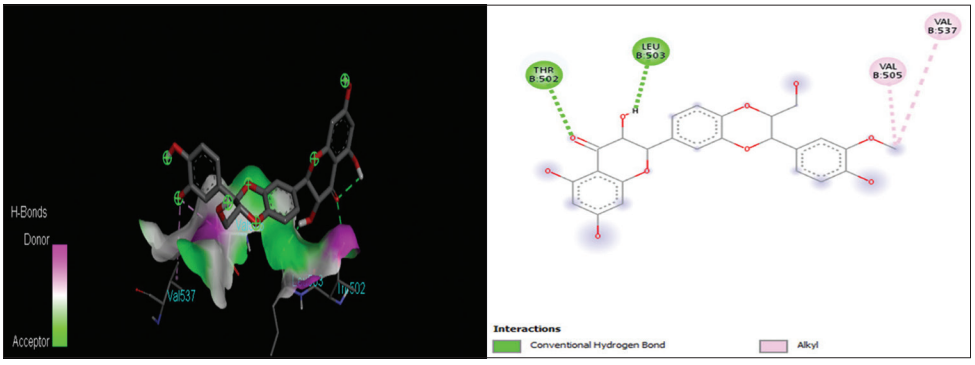

Figure 4: 3D and 2D interactions of silybin with α-glucosidase

of a number of synthetic oral antidiabetic drugs in the market, researchers have now diverted their attention to different herbs and medicinal plants to find out natural active phytochemicals with fewer side effects and better antidiabetic activity. Computational tools have increasingly become effective in inferring interactions between protein and chemical compounds after the genomic era. Through an *in silico* approach, the study aims to understand that *B. rotunda* flavonoids act on the human glucose control. Among all constituents of *B. rotunda,* there are eight flavonoid representatives which include pinocembrin, quercetin, alpinetin, boesenbergin, panduratin A, pinostrombin, silybin, and geraniol.

In the present study, the molecular mechanisms of inhibitory action of phytochemicals *B. rotunda* against therapeutic targets of T2D, namely, alpha glucosidase, DPP4, and peroxisome

Table 1: Interactions of peroxisome proliferator-activated receptor gamma residues with ligands at receptor sites

S. No.	Ligands	Binding affinity∆G (Kcal/mol)	Amino acids involved	
			Hydrogen binding interactions	Hydrophobic binding interactions
1.	Alpinetin	–8.1	SER B: 139,	ILE B: 138
2.	Boesenbergin A	–6.9	ARG B: 240	THR A: 237
3.	Geraniol	–5.6	LEU A: 25, GLU A: 92	----
4.	Panduratin	–7.2	ARG B: 240, THR B: 244	----
5.	Pinocembrin	–8.4	GLY A: 192, GLN A: 207, SER A: 191	----
6.	Pinostrombin	–7.8	GLY A: 192, GLN A: 207, ASP A: 193	----
7.	Quercetin	–7.8	GLU B: 140	----
8.	Silybin	-	TYR B: 117, ARG B: 194, ARG B: 240, ASP B: 193	ASP A: 238

Table 2: Interactions of dipeptidyl peptidase 4 residues with ligands at receptor sites

S. No.	Ligands	Binding affinity∆G (Kcal/mol)	Amino acids involved	
			Hydrogen binding interactions	Hydrophobic binding interactions
1.	Alpinetin	–6.8	ASP A: 588	ILE A: 375
2.	Boesenbergin A	–6.3	ALA A: 306	PRO A: 218, HIS A: 363
3.	Geraniol	–3.8	CYS A: 301	----
4.	Panduratin	–6.3	MET A: 348	ILE A: 375
5.	Pinocembrin	–7.2	ARG A: 382, GLU A: 347	VAL A: 354
6.	Pinostrombin	–7.1	GLY A: 355, SER A: 349, ASP A: 588	----
7.	Quercetin	–7.3	ARG A: 596, GLN A: 320, GLU A: 668	----
8.	Silybin	–8.2	MET A: 348, ILE A: 346, TRP A: 353, CYS A: 394	----

Table 3: Interactions of α-glucosidase residues with ligands at receptor sites

S. No.	Ligands	Binding affinity ∆ G (Kcal/mol)	Amino acids involved	
			Hydrogen binding interactions	Hydrophobic binding interactions
1.	Alpinetin	–3.4	----	----
2.	Boesenbergin A	–2.4	GLU B: 506	----
3.	Geraniol	–1.8	----	----
4.	Panduratin	–3.1	----	VAL B: 505
5.	Pinocembrin	–3.4	GLU B: 506	----
6.	Pinostrombin	–3.7	----	VAL B: 505
7.	Quercetin	–3.6	----	VAL B: 505
8.	Silybin	–3.7	THR B: 502, LEU B: 503	----

proliferator-activated receptor gamma (PPARG) were studied through molecular docking. All these phytochemicals follow the drug-likeness rules and the LogS as well as lethal factor concentration (LD50). On the basis of docking scores, hydrogen bond length between amino acids and ligands and the interaction, all phytochemicals showed good binding energy with all three receptors of T2D and they may be better ligands of respective targets. Out of all reference compounds, four phytochemicals are found potential antidiabetic drug candidates.

Out of all ligands, pinocembrin exhibits highest binding affinity of −8.4 Kcal/Mole which is free of amino acid residues interacting with hydrogen bonds and the docking interaction results of the ligands with PPARG are represented in Table 1. Silybin exhibits highest binding affinity of −8.1 Kcal/Mole and shows hydrogen bond interactions with MET A:348, ILE A:346, TRP A:353, and CYS A:394. The docking interaction results of the ligands with DPP4 are represented in Table 2. Silybin and alpinetin exhibit highest binding score of −3.4 Kcal/Mole and shown hydrogen bond

Table 4: Drug-likeliness

Physicochemical properties of ligands in accordance with the rules of drug-likeliness

Ligand	MW	LogP	MF	HBA	HBD	TPSA	Vol	nRB	MR	D	nA
Alpinetin	96.08	0.98	$C_5H_4O_2$	2	0	30.21	84.59	1	24	0	11
Boesenbergin A	122.12	1.85	$C_7H_6O_2$	2	1	37.30	111.05	1	33.4	0	15
Geraniol	118.14	2.26	C_8H_6O	1	0	13.14	109.60	0	36.2	0	15
Panduratin	150.18	2.13	$C_9H_{10}O_2$	2	1	29.46	145.34	2	44.7	0	21
Pinocembrin	154.16	1.34	$C_8H_{10}O_3$	3	1	38.70	143.15	2	37.5	0	19
Pinostrombin	168.24	3.08	$C_{10}H_{16}O_2$	1	1	37.30	177.76	4	50	0	28
Quercetin	148.16	1.62	$C_9H_8O_2$	2	0	37.70	177.76	4	41.9	0	19
Silybin	148.16	1.91	$C_9H_8O_2$	1	1	37.70	138.46	2	43.1	0	19

MW: Molecular weight; MF: Molecular formula; HBA: Hydrogen bond acceptor, HBD: Hydrogen bond donor; Vol: Volume; nRB: No. of rotatable bonds; MR: Molecular refractivity; D: Deviations; nA: No. of atoms

Table 5: Bioactive scores of ligands

S. No.	Ligands	GPC ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1.	Alpinetin	0.02	−0.23	−0.28	0.34	−0.16	0.17
2.	Boesenbergin A	0.03	−0.00	−0.16	−0.42	0.08	0.34
3.	Geraniol	−0.60	0.07	−1.32	−0.20	−1.03	0.28
4.	Panduratin	0.06	0.04	−0.24	0.35	−0.03	0.19
5.	Pinocembrin	−0.00	−0.20	−0.32	0.37	−0.17	0.21
6.	Pinostrombin	−0.04	−0.29	−0.32	0.30	−0.17	0.14
7.	Quercetin	−0.06	−0.19	0.28	0.36	−0.25	0.28
8.	Silybin	0.07	−0.05	0.01	0.16	0.02	0.2 3

Table 6: ADME

Ligands	LogS	$Caco_2$	HIA	Pg-S	PPB	BBB	V_D	CYP 450						T	CL
								1A2	2C19	2C9	2D6	3A4	2D6S		
Alpinetin	−3.23	−4.18	0.873	0.041	50.177	4.29	−0.281	0.063	0.31	0.977	0.498	0.315	0.432	1.311	1.6
Boesenbergin A	−5.88	−4.193	0.899	0.014	40.811	4.41	−1.039	0.00	0.09	0.021	0.28	0.039	0.156	1.119	1.146
Geraniol	−2.62	−4.066	0.924	0.058	48.597	4.25	0.108	0.011	0.26	0.98	0.656	0.547	0.473	1.385	1.815
Panduratin	−5.88	−4.55	0.722	0.032	55.734	4.01	−0.099	0.062	0.39	0.802	0.488	0.306	0.776	1.084	1.678
Pinocembrin	−3.18	−4.143	0.808	0.041	28.026	3.74	−0.376	0.003	0.29	0.907	0.58	0.29	0.324	0.899	1.276
Pinostrombin	−3.21	−4.446	0.757	0.01	68.741	4.30	−0.669	0.003	0.12	0.034	0.126	0.178	0.378	1.616	1.138
Quercetin	−2.19	−4.709	0.764	0.047	30.415	2.55	−0.26	0.005	0.39	0.482	0.65	0.482	0.821	1.392	1.602
Silybin	−2.24	−4.386	0.895	0.059	46.371	1.21	−1.212	0.001	0.16	0.082	0.432	0.117	0.282	1.345	1.012

Table 7: ADME

S. No.	Pharmacokinetic property	Preference	Predictions for all ligands
1.	Log S (water solubility)	<10	Low solubility
2.	$CaCo_2$ permeability	4.0–5.5	Optimal permeability
3.	HIA (human intestinal absorption)	HIA ≥ 30% – category 0 HIA+<30% – category 1	Belongs to both the categories 0 and 1
4.	P-glycoprotein substrate	P^{ka}<8, MW<400	P-gp Non-substrate
5.	PPB (plasma protein binding)	90	Optimal
6.	BBB permeability	BBB score>4	All are Permeable
7.	V_D (volume distribution)	0.04–20	Optimal
8.	CYP4501A2 inhibitor	Category 0: Non-inhibitor/ Non-substrate Category 1: Inhibitor/Substrate	Non-inhibitor
9.	CYP4502C19 substrate		Substrate
10.	CYP4502C9 inhibitor		Non-inhibitor
11.	CYP4502D6 substrate		Non-substrate
12.	CYP4502D6 inhibitor		Non-inhibitor
14.	$T_{1/2}$ (half-life)	>0.5–3 h	Low
15.	CL (clearance)	<5 ml/min/kg	Low

interactions with THR B:502, LEU B:503 of DPP4. The docking interaction results of the ligands with α-glucosidase are represented as Table 3.

The key amino acids of PPARG involved in the interaction are GLY A:192, GLN A:207, and SER A:191. The amino acids of DPP4 involved in the interaction are MET A:348, ILE A:346, TRP A:353, CYS A:394. THR B:502, and LEU B:503 which are the amino acids involved in the interaction with α-glucosidase. On the basis of pharmacophore studies, pinocembrin, alpinetin, and silybin have common physicochemical properties and high selectivity scores as compared to antidiabetic drugs such as metformin, rosiglitazone, and sitagliptin. All the ligands are blood–brain barrier permeable and have low clearance rate <5 ml/kg/min with low solubility and less half-life [Tables 4-7].

CONCLUSION

The phytoconstituents involved in the study have the following antibacterial, anti-inflammatory, and hepatoprotective properties. *B. rotunda* polyphenols are expected to be involved in the pathophysiology of diabetes. The polyphenol and flavonoid family had a high binding affinity for all three receptors. All ligands show good pharmacokinetics. Pinocembrin, silybin, and alpinetin show a very high binding affinity with PPARG, DPP4, and α-glucosidase and show the natural activities used to treat diabetes. The following study demonstrated from an *in silico* approach that polyphenols and flavonoids found in *B. rotunda* have the potential to interact and modulate the activity of proteins involved in the main pathways of T2D. Nevertheless, further investigations with different animal models and humans should be carried out to confirm these findings and gain a better understanding of the mechanisms of these polyphenols underlying their metabolic health properties.

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