Stevia rebaudiana Bertoni leaf extract phytochemicals inhibit the Type 2 Diabetes mellitus receptor targets

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Article

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Abstract

*Stevia rebaudiana* Betoni (SR) or the candy leaf herbal plant is widely consumed as a non-caloric natural sweetener in substitute of table sugar. The perennial plant is native to South America and is cultivated across the globe. Over the years, there has been an increasing scientific interest in Stevia leaves, which naturally inherent phytochemicals of potent pharmacological properties. Amongst the various pharmacological activities previously evident through *in vitro* or *in vivo* studies includes blood sugar regulators, antibacterial, and antiviral properties. Type 2 diabetes mellitus (T2DM), or adult-onset diabetes, is a chronic condition characterized as non-effective insulin production by the pancreas. As a result, blood sugar rises and leads to fatality under an extremely high blood sugar condition. In this study, a library of SR leaf phytochemicals was screened using a comprehensive computational approach for the identification of potential anti-T2DM drug compounds. The *in silico* inhibitory activities of SR phytochemicals against selected T2DM receptor targets (T2DMrTs) were measured by molecular docking analysis and the best complex, as indicated by the minimum binding affinity and extent of structural interactions, was validated further by molecular dynamics (MD) simulation using the GROMACS webserver. The SR phytochemicals were searched in open-source databases and the corresponding pharmacological properties were predicted by the SwissADME tool. The following proteins were set as the T2DMrTs: α-amylase, α-glucosidase, DPP-4, GLP-1R, PPAR-γ and PTP1B. A total of 177 SR phytochemicals were mined and further screening by molecular docking analysis and pharmacological evaluation identified 19 compounds satisfying the minimum binding energy set at < -7 kcal/mol, Lipinski’s rule of five, and oral bioavailability score. Under all possible receptor-ligand, pair-wise combinations, the complexes showed strong to good stability under a 50 ns MD simulation run. The findings inform new information on SR phytochemicals for T2DM therapeutics and drug development.

Introduction

Diabetes mellitus (DM) is a globally prevalent non-communicable metabolic disease¹, characterized as plasma glucose level disorder (abnormally high blood sugar). The disease starts mostly in between the middle-to-late adulthood, however, young children and teenagers are developing the condition alike. Type 2 Diabetes mellitus (T2DM), also known as non-insulin dependent diabetes mellitus (NIDM), is the most common form of DM (> 90% of DM occurrence)²,³. The human cells resist insulin, leading to insulin resistance and glucose build up in the blood. Over time, DM damages blood vessels and favours numerous pathological disorders such as kidney disease, vision loss, and cardiovascular diseases⁴. According to the International Diabetes Federation, the global DM prevalence was recorded at 463 million people in 2019. It is projected that the number is likely to increase to 578 million by the next decade and leap up further to 700 million by 2045⁵.

Majority of T2DM patients are prescribed with drugs to control and sustain an optimum blood glycemic index. Oral metformin (Fortamet and Glumetza) is the most preferred go-to-go drug in T2DM treatment, especially for the pre-diabetes condition⁶. Despite improving the body cells’ sensitivity, the oral metformin
prescription has been associated with irregular bowels and abdominal discomfort side effects. Others among the sulfonylureas (glyburide, glimepiride and glipizide), glinides and thiazolidinediones groups of the T2DM drugs, a significant weight gain is most evident apart from other possible side effects. In advanced conditions, often treated with injectable medications such as the DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT2 inhibitor, patients encounter adverse side effects: risk of pancreatitis, bone fractures, amputation, joint pain, and low blood pressure.

Natural products and traditional medicines offer an alternative route to the current synthetic drug prescription in T2DM treatment. Plants are an excellent source for drug development as they pose minimal side effects to the human body. There are about 200 thousand natural products with well-documented structural information, of which, the value represents only 15% of the total plant species, estimated at 350 thousand plant species. Plant-based medicines represent a third of FDA-approved drugs.

Under this context, Stevia rebaudiana Bertoni (SR), a phytochemical powerhouse herbal plant, serves as an excellent candidate for T2DM drug development. The SR extract have been reported to display anti-hypertensive, anti-hyperlipidemia, anti-diabetic, antioxidant, anti-hypertensive, antimicrobial, anti-inflammatory, anticarcinogenic, anti-obesity and anti-hyperglycemic activities. There are about 230 species in the genus Stevia, however, the sweet essence enriched phytochemicals are specifically present in the SR only. The SR phytochemicals are almost 300 times sweeter than the table sugar, and thus, the SR leaves are consumed as natural sweeteners across the world. The concentrated form is manufactured in the food industry and in non-alcoholic beverages.

The primary patho-physiological correction in DM management is to reverse the elevated blood glucose concentration condition. There are various DM receptor targets identified in DM disease development: i) α-amylase, ii) α-glucosidase, iii) dipeptidyl peptidase-4 (DPP-4), iv) glucagon-like peptide 1 receptor agonists (GLP-1R), v) peroxisome proliferator-activated receptor-gamma (PPAR-γ), and vi) protein tyrosine phosphatase 1B (PTP1B). Pancreatic α-amylase and intestinal α-glucosidase are involved in the onset of T2DM development. Inhibiting the mechanistic activities of pancreatic α-amylase and α-glucosidase is an important strategy in delaying carbohydrate digestion and the postprandial hyperglycemia condition in T2DM patients. The dipeptidyl peptidase-4 (DPP-4) is a key target in T2DM treatment. It is ubiquitously enzyme that inactivates glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), causing a loss in insulinotropic activity. The glucagon-like peptide 1 (GLP-1) receptor suppresses the release of glucagon and augments insulin secretion at physiological plasma glucose concentrations after carbohydrate meals. The insulinotropic potential of GLP-1R agonists is also another key receptor target in T2DM treatment.

Peroxisome proliferator-activated receptor gamma (PPAR-γ) is a significant modulator of obesity and T2DM. PPAR-γ acts as a master regulator of gene expression networks involved in adipocyte differentiation, lipid metabolism, glucose homeostasis, inflammation and cell proliferation in humans.
In skeletal muscles, PPAR-γ receptor agonists augments insulin sensitivity by triggering muscle glucose uptake and supressing liver glucose release. The activation of PPAR-γ significantly improves insulin sensitivity and combats hyperglycaemia in T2DM\(^2, 26\). Protein tyrosine phosphatase 1B (PTP1B), an enzyme from the PTP family, is primarily involved in insulin and leptin signalling regulation\(^27\). PTP1B enzyme highly expressed in liver, muscles and fat, down-regulates insulin signalling by catalysing the dephosphorylation of activated insulin receptor and insulin receptor substrate\(^28, 29\). The inhibition of PTP1B mitigates insulin resistance, resulting from PTP1B over activity. Thus, PTP1B is a promising and effective therapeutic approach in the treatment of T2DM and excessive weight gain\(^30, 31\).

Computer-aided drug discovery (CADD) approach is an emerging tool deployed in drug discovery against various diseases. High-throughput computational methods are rapid, efficient, cost-effective, and minimizes the negative risk in drug development pipelines prior to clinical trials\(^32\). In this study, the SR phytochemicals (ligand) were screened for potential inhibitory activities against key T2DM receptors. The SR phytochemicals were evaluated thoroughly using input data fetched from open-source databases. The receptor-ligand complexes with promising stability, as indicated by the molecular docking and pharmacology network analyses were validated by molecular dynamics simulation.

**Materials And Methods**

**Construction of *Stevia rebaudiana* (SR) phytochemical library**

A bibliomic search on disease related to SR was performed using the Disease List Automatically Derived For You (DLAD4U) server (http://dlad4u.zhang-lab.org/index.php)\(^33\) with “*Stevia rebaudiana* Bertoni”, set as the query term. To generate a library of SR leaf phytochemicals, the following databases were accessed: TCMSP (Traditional Chinese Medicine Database and Analysis Platform; https://tcmsp-e.com/), ETCM (The Encyclopedia of Traditional Chinese Medicine; http://www.tcmip.cn/ETCM), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov), and HERB (a high-throughput experiment- and reference-guided database; http://herb.ac.cn/). The SR phytochemicals were also identified from literatures available in the following search engines: i) Google Scholar (www.scholar.google.com) ii) Scopus (www.scopus.com) and iii) Clavariate of Analytics Web of Science (www.webofknowledge.com).

**Ligand pre-processing**

The SDF files for each of the SR phytochemicals along the following standards were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/): acarbose (PubChem ID: 41774) for α-amylase and α-glucosidase, rosiglitazone (PubChem ID: 77999), Decyl 4-O-alpha-D-Glucopyranosyl-1-Thio-beta-D-Glucopyranoside (PubChem ID: 10345813) and 2-(Oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-C]pyridine-3-carboxylic acid (PubChem ID: 444766). The SDF files were converted into PDBQT files using OpenBabel available in PyRx software\(^34, 35\). The ligand optimization was performed using the MMFF94 force field followed by 1000 steps, energy minimization and conjugate-gradients algorithm\(^36\). The chemical
structure of each target compound was drawn using Chem-Space (https://chem-space.com/), an online chemistry tool.

Type 2 Diabetes mellitus receptor targets (T2DMrTs) pre-processing

The following T2DMrTs were designated as molecular targets of T2DM: α-amylase, α-glucosidase, dipeptidyl peptidase-4 (DPP-4), glucagon-like peptide 1 receptor (GLP-1R), peroxisome proliferator activated receptor gamma (PPAR-γ), and protein tyrosine phosphatase 1B (PTP1B). The functional annotation of the designated receptors (T2DMrTs) were confirmed by literature search and public databases: Drugbank database (www.drugbank.ca) and Therapeutic Targets Database (http://db.idrblab.net/ttd/). The X-ray crystallographic structures of α-amylase (PDB ID: 1HNY), α-glucosidase (PDB ID: 3A4A), DPP-4 (PDB ID: 2RIP), GLP-1R (PDB ID: 6LN2), PPAR-γ (PDB ID: 3DZY), and PTP1B (PDB ID: 6B8Z) were retrieved from the Protein Data Bank (www.rcsb.org). Each individual receptor was manually edited, curated, and refined using AutoDockTools 4.2, Pymol tool version 2.5.2, and Modloop server (https://modbase.compbio.ucsf.edu/modloop/); non-amino acid residues were discarded and polar hydrogen and Kollman charges were added.

Prediction of druggable pockets

For structure-based molecular docking, druggable pockets of each T2DmMT receptor was identified by the PrankWEB server (https://prankweb.cz/). This computational tool predicts the druggable pockets and generates scores from protein structures by utilizing template-independent machine learning approach. The top-ranked predicted pockets were selected and used in the molecular docking analysis. (Table 1 and Supplementary Table S1).

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Molecular docking

Molecular docking was performed using AutodockVina tool: predicts the magnitude of protein-ligand interaction in its scoring function (binding affinity). Docking grids of all macromolecule receptors, were
adjusted into squares of 30 Å with x, y, and z coordinates to define the binding sites (Table 1). For each protein-ligand complex, the minimum binding energies expressed in kcal/mol was measured at RMSD = 0. The interactions of the protein-ligand complex were visualized using the BIOVIA Discovery Studio Visualizer version 20.1.0 and ChimeraX version 1.4 tool \(^{45,46}\). The following compounds were selected as reference ligand molecules; acarbose for α-amylase and α-glucosidase, rosiglitazone for DPP-4 and PPAR-γ, Decyl 4-O-alpha-D-Glucopyranosyl-1-Thio-beta-D-Glucopyranoside for GLP-1R, and 2-(Oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-C]pyridine-3-carboxylic acid for PTP1B.

Screening of SR phytochemicals’ pharmacological properties.

The following pharmacological properties of SR phytochemicals were evaluated using the SwissADME server (http://www.swissadme.ch/): i) ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity), ii) oral bioavailability (OB), and iii) molecular information \(^{47}\).

Network construction

The T2DMrTs were retrieved from Uniprot (www.uniprot.org) database. The SR phytochemical-T2DMrT receptor network was constructed using Cytoscape version 3.7.2 (https://cytoscape.org/) \(^{48}\). The pharmacological network was used to identify the hub clusters comprised of SR phytochemicals with the most number of connections with T2DMrTs.

Molecular dynamics (MD) simulation

A MD simulation run of 50 ns was performed on selected receptor-ligand complexes using the GROMACS simulation tool, the WebGro webserver (https://simlab.uams.edu/index.php). The ligand topology was prepared using the PRODRG server (http://prodrg1.dyndns.org/) \(^{49}\). The receptor-ligand complex was subjected to GROMOS96 43a1 force field solvated in the Single Point Charge (SPC) water model, fixed in a periodic cubic solvated box and 0.15 M of NaCl was added to neutralize the system. The system was energy minimized with 5,000 steps of the steepest descend algorithm at 300 K and 1 bar of pressure under an NVT/NPT ensemble with a leap-frog MD integrator. The trajectory analyses of the ligand-protein complex was evaluated by the following parameters: the average number of hydrogen bonds, root mean square deviation (RMSD), and root mean square fluctuation (RMSF).

Results

*Stevia rebaudiana* (SR) phytochemical library characterization

The keyword (*Stevia rebaudiana*) search via DLAD4U server revealed the highest hit score for the following descriptions: i) taste, ii) T2DM, iii) weight gain, and iv) insulin resistance. The comprehensive annotation of DLAD4U output is presented in Supplementary table S2. Of the four phytochemical databases accessed in search of SR phytochemicals, matching hits were obtained from the Dr. Duke phytochemical and ethnobotany database only. Identical compounds were filtered off and a library of
177 different SR phytochemicals were obtained. A total of 80 phytochemicals were obtained from Dr. Duke's server, and the balance were collected from bibliomic search on scientific literatures\textsuperscript{50–54}. The SR phytochemicals are presented in Supplementary table S3, and their previously reported antidiabetic potential is documented in Supplementary table S6.

Virtual screening based on molecular docking.

Molecular docking of 177 ligands (SR phytochemicals) with six T2DMrTs under all possible combinations was performed in parallel with reference ligands. The minimum binding affinity (MBA) of the target compound and the reference compound against the T2DMrTs are presented in Supplementary table S4. An upper threshold at MBA=-7 kcal/mol was set for the screening of stable receptor-ligand complex\textsuperscript{55}. Complexes that meet the requirement of MBA<-7 kcal/mol were selected for subsequent analysis. Of the 177 SR phytochemicals (T2DMrT-SR phytochemical complexes), a total of 109 compounds showed good stability with the six T2DMrTs under various combinations: 135 with α-glucosidase, 106 with DPP-4, 98 with GLP-1R, 87 with PPAR-γ, and 80 with PTP1B (Supplementary Table S5).

The MBA of all the SR phytochemical-bound α-amylase complexes ranged at -10.2 to -4.5 kcal/mol (Supplementary Table S4). Rebaudioside E-, rebaudioside F-, β-amyrin acetate-, and rebaudioside A- α-amylase complexes showed the least MBA at -10.2 kcal/mol, -10.1 kcal/mol, -10 kcal/mol, and -9.9 kcal/mol respectively, greater than acarbose positive control (MBA=-7.1 kcal/mol) (Supplementary Table S5). The MBA of all phytochemicals complexed with α-glucosidase ranged at -11.4 to -5.4 kcal/mol. Apigenin 4’-O-glucoside-, 3,4,5-tricaffeoylquinic acid- and luteolin-7-O-glucuronide- bound α-glucosidase complexes exhibited the least MBA at -11.4 kcal/mol, -11.2 kcal/mol, and -11 kcal/mol, respectively. The MBA values were stronger than the acarbose- α-glucosidase, positive control complex (MBA= -8.5 kcal/mol) (Supplementary Table S4 and S5). In DPP-4- bound SR phytochemical complexes, the MBA ranged at -10.3 to -4.6 kcal/mol (Supplementary Table S4). Dulcoside A-, 3,4,5-tricaffeoylquinic acid-, and rebaudioside F- bound DPP-4 complexes showed the least MBA at -10.3 kcal/mol, -10 kcal/mol, and -10 kcal/mol, respectively, comparatively greater than the rosiglitazone- DPP-4, reference complex (MBA= -8.0 kcal/mol) (Supplementary Table S5). The MBA of SR ligand bound-GLP-1R complexes ranged at -10.3 to -4.6 kcal/mol. Rebaudioside C-, β-amyrin acetate-, and rebaudioside F- bound GLP-1R complexes showed the least MBA at -10.3 kcal/mol, -10 kcal/mol, and -9.9 kcal/mol respectively, which was greater than the decyl 4-O-alpha-D-glucopyranosyl-1-thio-beta-D-glucopyranoside, positive control complex (MBA= -7.0 kcal/mol) (Supplementary Table S4 and S5). The MBA values of all 177 phytochemicals with PTP1B ranged at -9.6 to -4.0 kcal/mol. Rebaudioside C-, rebaudioside F-, and rebaudioside A- PTP1B bound complexes showed the least MBA at -9.6 kcal/mol, -9.6 kcal/mol, and -9.1 kcal/mol, respectively. The MBA values of the target compounds was greater than the 2-(Oxalyl-amino)-4,5,6,7-tetrahydro-thieno[2,3-C]pyridine-3-carboxy, reference compound (Supplementary Table S4 and S5). A ligand-receptor amino acid interaction sites of isosteviol (ISV) with all selected T2DMrTs are demonstrated in Fig. 1.
The SR phytochemicals from the T2DMrT-bound complex at MBA<-7 kcal/mol showed good bioavailability (Abbot Bioavailability score) and met the Lipinski rule of five on independent evaluation. A network was constructed using SR phytochemicals that passed the minimum binding values and fulfilled the pharmacological properties. The network identified hub SR phytochemicals, which showed association with all the six different T2DMrTs. The 19 SR phytochemicals (yellow square) were connected to all the different receptors (pink triangle) in all possible combinations (Fig. 2).

Molecular Dynamic (MD) Simulation

From the 19 SR phytochemicals, isosteviol (ISV) was selected for MD simulation validation. The trajectory analysis of the following complexes were evaluated: i) α-amylase-ISV, ii) α-glucosidase-ISV, iii) DPP4-ISV, and iv) PTP1B-ISV. The root-mean-square-deviation (RMSD) of all the six complexes ranged at 0.2–0.6 nm (Fig. 3).

The root mean square fluctuation (RMSF) analysis was conducted to analyze residue-wise fluctuations of core amino acids of the receptor protein. The high RMSF values of amino acid residues suggest that the receptor is mobile and unstable. The lower the RMSF values for amino acid residues, the more stable and rigid the receptor protein. Throughout the MD run, the RMSF for all the six protein receptor complexes was in the range of 0.1 to 0.4 nm. There was no notable deviation that occurred in the protein regions for all the complexes, indicating the reason for stable RMSD plots for all the substrate ligand-bound complexes (Fig. 3).

Upon MDS analysis, it has been seen that the ligand-protein complexes exhibit an increased number of H-bonds, which is expected to strengthen the interaction of the complex and, subsequently, make the complex highly stable. Throughout the MD simulation, the total number of all the receptor-ligand (ISV) intermolecular hydrogen bonds remained stable at roughly 1 to 4 (Fig. 3). These results indicate that the ligand (ISV) was retained in the docking pockets of the respective receptor proteins. Briefly, the ISV-α-glucosidase complex, showed three stable H-bonds conspicuously after 10 ns and onward MD run. The DPP4-ISV complex showed similar results, with approximately 1 stable H-bond and two dynamic ones showing stability after 2.5 ns. The remaining four complexes, i.e., α-amylase, GLP-1R, PPAR-γ, and PTP1B have average hydrogen bond tendencies of one stable H-bond throughout the MD run (Fig. 3).

Discussion

Natural products have a long history in the treatment of various acute and chronic diseases. In the recent time, an ever growing disease treatments formulated with plants’ active ingredients have been deployed massively. A wide variety of phytochemicals are used as therapeutic drugs for various ailments, including but not limited to infectious diseases, diabetes, obesity, inflammation, heart disease, neurological disorders, and many types of cancer. Natural products constitute a pool of phytochemicals with known and unknown functions, otherwise addressed as unleashed therapeutic potentials. Screening of these bioactive compounds using the conventional method (laboratory scale experiments) is laborious.
and time consuming, taking into account the enormous size of phytochemical library and plant species biodiversity\(^{46}\).

In others, independent phytochemical, singly had demonstrated positive interactions with multiple disease targets. For instance, genistein exerts inhibitory interactions with different receptors corresponding to diabetes, cancer, inflammation and others \(^{58}\). Others such as salidroside, luteolin, esculin, xanthorrhizol, curcumin, costunolide and narirutin have also demonstrated pharmacological potential against multiple protein targets of various diseases \(^{59-66}\). The SR leaves’ nutritional value as well as medicinal potential is underrated. Large number of previous research studies have reported that SR has a variety of therapeutic potentials, including T2DM treatment\(^{10-12}\).

In the present study, a library of 177 SR (leaves) phytochemicals was screened against T2DM using comprehensive computational tools. Based on the molecular docking and pharmacological properties screening, a total of 19 SR phytochemicals were shortlisted: ethyl chlorogenate isomer 1, isosteviol, 3-O-caffeoylshikimic acid, sterebin N, salidroside, diosmetin, 5-p-coumaroylquinic acid, austroinulin, luteolin, genistein, sterebin E, 4-O-caffeoylshikimic acid, 4-coumaroylquinic acid, sterebin B, 3b,12a-dihydroxy-5b-cholanic acid, steviolmonoside, 3-coumaroylquinic acid, esculin, and 6-O-acetylaustroinulin. The shortlisted SR phytochemicals showed a minimum binding affinity score< -7 kcal/mol and met the Lipinski’s rule of five and OB criteria (Table 2).

Part of these 19 SR phytochemicals were previously annotated with antidiabetic potential; salidroside, luteolin, genistein, esculin, diosmetin, and isosteviol \(^{67-72}\). In an \textit{in vivo} study conducted by Nordentoft et al. (2008), isosteviol (SR phytochemical) improved glucose homeostasis, enhanced insulin sensitivity and up-regulated beta-cell genes (GLUT2, Ins1, Ins2) in diabetic KKAy mouse\(^{72}\). Similarly, Venkateshwarlu et al. (2015) also revealed the antidiabetic potential of esculin through an \textit{in vivo} study\(^{70}\). Fu et al. (2010) showed that genistein markedly improved hyperglycemia, blood insulin level, and improved pancreatic islet \(\beta\)-cell proliferation in streptozotocin-induced diabetic mice\(^{69}\). In brief, the results of the present study agreed with previous studies that were carried out independently on salidroside, luteolin, genistein, esculin, diosmetin, and isosteviol.

In drug discovery and design, pharmacological properties screening based on Lipinski’s rule of five and the Abbot Bioavailability Score (OB) score are important criteria to evaluate the probable drug candidates\(^{46,73}\). Lipinski’s rule determines the drug-likeness properties of a compound as following: molecular weight (MW) \(\leq 500\), Log P \(\leq 5\), number of hydrogen bond acceptors and donors less than 10 and 5, respectively\(^{74,75}\). The OB score predicts the chance of absorption and oral intake bioavailability at the site of drug action. Higher OB (cut-off value = >10%) scores correspond to a greater drug potency upon oral administration\(^{55}\). The pharmacokinetic properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) predict the drug’s absorption into the blood stream upon oral consumption and to avoid interference with the central nervous system\(^{55}\).
From the 19 SR phytochemicals investigated in this study, isosteviol (ISV) was selected for validation by molecular dynamic (MD) simulation. ISV fulfilled all the Lipinski’s rule of five and numerous previous studies have also reported the antidiabetic potentials of ISV\textsuperscript{72,76}. Through MD simulation, the stability and dynamic behavior of all six selected receptors with ISV, under all ISV-receptor pair wise combination were evaluated in an aqueous environment for 50 ns (Fig. 3). The trajectory analysis of the MD simulation run was analysed by root mean square deviation (RMSD), root mean square fluctuation (RMSF) and the number of hydrogen bonds. The RMSD inferred the extent of deviation held by the ISV-bound receptor complexes whereas the RMSF measured the displacement of the group atoms relative to the reference structure.

The RMSD values of all ISV-bound α-amylase, α-glucosidase, DPP4, and PTP1B complexes stably fluctuated within minimal fluctuation at 0.2–0.35 nm. The result suggests that the ligand molecule was contained within the receptor binding pocket region cavity through a strong and stable interaction between the receptor residue (RR) and ligand atoms (Fig. 3).

In this study MD simulation, a moderately reliable approach was employed for validation. It is essential to perform functional assays in cell lines or \textit{in vivo} studies for a successful up-stream discovery of drugs. Despite these limitations, the comprehensive computational study presented here provides a critical, fast, and cost effective evaluation of the phytochemical profile of SR and yields valuable information that can be applied in wet lab experiments. The current findings inform therapeutic potential of SR compounds against T2DM and will require further laboratory-scale experimental validations.

**Conclusion**

In this study, \textit{Stevia rebaudiana} Bertoni (leaves) phytochemicals were screened against selected antidiabetic therapeutic targets (α-amylase, α-glucosidase, DPP-4, GLP-1R, PPAR-γ, and PTP1B) using a comprehensive computational analysis. A library 177 phytochemicals was filtered based on the minimum binding affinity values, Lipinski’s rule of drug-likeness and oral bioavailability properties. A total of 19 phytochemicals passed the minimum requirement and one compound was validated further by molecular dynamic simulation. Isosteviol subjected to MDS with all six receptor proteins showed stable trajectory under a 50 ns run. This study reveals a list of candidate bioactive drugs from SR phytochemicals highly fitted for the development of antidiabetic drugs.

** Declarations**

**Acknowledgement**

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Author contributions

M.S write the initial draft of the manuscript, performed the experiments and analyzed the data. A.A, M.Z, N.H.K, N.G, A.A.N, D.L, and S.F. equally contributed in writing and analyse the data for the manuscript. S.F and D.L, provide the main conceptual ideas and proof outline of the manuscript.

Conflict of interest

The authors declare no competing interests.

Data availability

All data generated or analysed during this study were included in this article.

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72. Nordentoft, I., Jeppesen, P. B., Hong, J., Abudula, R. & Hermansen, K. Isosteviol increases insulin sensitivity and changes gene expression of key insulin regulatory genes and transcription factors in


Table 2 is available in the Supplementary Files section.

Figures

![Figure 1](image-url)
Three-dimensional (3D) illustration of isosteviol (ISV) with selected target protein targets (a-f). In receptor-ligand complexes, the brown area represents the interaction sites of the ligands. The magenta colored compound represented by the crystal stick model is isosteviol (ISV), and the yellow-colored compounds are the compounds that were run as reference positive controls in molecular docking simulation. In addition, the localization of active amino acid residues with the types of interaction bonds of all T2DMrTs-ISV bound complexes has also been shown in 2D interaction.

Figure 2

A drug-target network was constructed using Cytoscape software. The green-colored nodes are all the active compounds which were shortlisted after molecular docking and pharmacological screening analyses. The yellow-colored nodes are all the hub bioactive compounds that were found to be common in all sets of the target proteins (magenta color node).
Figure 3

Receptor-ligand interactions over the period of MD simulation. RMSD, RMS fluctuation and number of hydrogen bonds of protein backbone and in complex forms of; (a) α-amylase-ISV, (b) α-glucosidase-ISV, (c) DPP-4-ISV, (d) GLP-1R-ISV, (e) PPAR-γ–ISV, and (f) PTP1B-ISV.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx
- TableS1.xlsx
- TableS2.xlsx
- TableS3.xlsx
- TableS4.xlsx
- TableS5.xlsx
- TableS6.docx
- TableS7.xlsx