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COMPUTATIONAL INVESTIGATION OF SELECTED PHYTOCHEMICALS AS POTENTIAL ANTIDIABETIC AGENTS TARGETING SODIUM/GLUCOSE TRANSPORTER2 (SGT2)

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ABSTRACT

The use of computer-aided methods in drug discovery, prediction of toxicity and pharmacokinetic profile is an area of research with increasing interest. This study was carried out in order to determine the pharmacokinetic profile, toxicity and potential to inhibit the SGT2 of Bacosine, bergenin, tiliroside and Swertiamarin using *in silico* tools. SwissADME server was used to determine their Blood brain barrier (BBB) permeation, Human Intestinal Absorption (HIA), P-glycoprotein substrate (P-gp), cytochrome P450 isoform inhibition, Skin permeation Log Kp and bioavailability score. Protox-II server was used to predict the organ toxicities and toxicological end points of the ligands and their LD50. AutoDock Vina was used for the docking studies. Bacosine and bergenin showed high human intestinal absorption (HIA) while Swertiamarin and tiliroside showed low HIA. All the compounds do not permeate the blood brain barrier (BBB). The compounds in this study are not substrates of permeability glycoprotein (P-gp). All the compounds in this study do not inhibit any of the CYP450 enzymes except Bacosine which inhibits CYP2C9. The compounds do not permeate the skin. Tiliroside and bergenin show immunotoxicity while Bacosine was active for carcinogenicity. Bacosine and tiliroside have lower binding energy (-9.1 kcal/mol and -11.0 kcal/mol respectively) than the standard drug Dapagliflozin. According to the findings of this study, Bacosine was found to have good pharmacokinetic profile, low toxicity and binding affinity higher than the standard drug. Bacosine may serve as a lead compound in discovery of new antidiabetic agents.

KEYWORDS: Phytochemicals, Pharmacokinetic profile, Sodium/glucose transporter 2, Computational investigation.

1. INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder caused by an absolute or relative lack of insulin and/or reduced insulin activity that results in hyperglycemia and abnormalities in carbohydrate, fat and protein metabolism.[1] The global prevalence of diabetes is on a steady rise and diabetes is one of the leading causes of morbidity and mortality worldwide.[2] The rise prevalence of Diabetes has aroused the need for development of many new approaches for management in order to maintain normal to prevent the development of complications.[3] Currently used oral hypoglycemic agents include; biguanides, sulfonylureas, meglitinides, Peroxisome-proliferator activated receptor- γ (PPAR-γ) agonists (glitazones), α-glucosidase inhibitors, Dipeptidyl peptidase-4 (DPP-4) inhibitors, Sodium/ glucose transporter 2 (SGLT2) inhibitors, dopamine-2 agonists.[4] These agents are expensive and have serious side effects.[5] Therefore the search for cheaper, safer and effective agents for the management of diabetes continues to be an important field of research.[6] The study of medicinal plants has led to the discovery of new chemicals for potential development as drugs that act on new or known therapeutic targets.[7] Numerous studies have demonstrated that natural product extracts and/or their active phytochemicals showed various antidiabetic properties, such as insulinotropic effect, peroxisome proliferator-activated receptor (PPAR) activation, AMP-activated protein kinase (AMPK) pathway activation, _-glucosidase inhibition, glucose transporter 4 (GLUT4) expression/translocation, and protein tyrosine phosphatase 1B (PTP1B) inhibition, with lower side effects.[8]

Sodium-dependent glucose co-transporters (SGLTs) are membrane proteins that are important molecular targets for drugs to treat diabetes and obesity.[9] Two most well characterized members of SGT family are SGT1 and SGT2.[10] SGLT2 is a low affinity and high capacity transporter that is found to be exclusively expressed in

the kidney proximal convoluted tubule and plays a significant role in renal glucose reabsorption. The inhibition of SGLT2 induces glycosuria and lowers blood glucose levels.[11] SGLT2 is therefore an attractive target for management of diabetes mellitus.[12]

Computational investigation involves the use of computer-based methods to help discover inhibitors with high binding capabilities with a protein target, drug-likeliness properties, and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) of small molecules. These methods have been used to discover various inhibitors for a spectrum of diseases [13, 14].

In this study, an attempt has been made to investigate the pharmacokinetics, toxicological profile and potential inhibition of Sodium/glucose transporter 2 of four phytochemicals namely; Tiliroside, bergenin, Swertiamarin and Bacosine using in silico methods.

2. MATERIALS AND METHODS

2.1. Molecular Properties and Bioactivity Scores of the ligands

Swiss ADME tool[15] was used to evaluate the molecular properties of the phytochemicals. Molecular properties such as LogP (partition coefficient between *n*-octanol and water), topological polar surface area (TPSA), the number of hydrogen bond donors and acceptors were estimated. Bioactivity Scores of the ligands of the selected phytochemicals (Tiliroside, bergenin, Swertiamarin and Bacosine) were investigated as potential G-protein coupled receptor (GPCR) ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI) and enzyme inhibitors (EI) using Molinspiration online server (http://www.molinspiration.com/).[16]

2.2. Pharmacokinetic Profile

SwissADME server was used to determine the pharmacokinetic parameters of Tiliroside, bergenin, Swertiamarin and Bacosine.[15] The parameters evaluated include; Blood brain barrier (BBB) permeation, Human Intestinal Absorption (HIA), P-glycoprotein substrate (P-gp), cytochrome P450 isoform inhibition, Skin permeation Log Kp and bioavailability score.

2.3. Prediction of Toxicity

Protox-II server[17] was used to predict the organ toxicities and toxicological end points of the ligands and their LD50. The integrated PubChem search (https:/pubchem.ncbi.nlm.nih.gov/) was used to search for chemical structures using the compound names. The models to be used were selected and the webserver computed the acute toxicity and toxicity targets selected.

2.4. Ligand preparation

The SDF format of tiliroside, bergenin, Swertiamarin and Bacosine were retrieved from www.zinc15.org. The ligands were imported into the Pyrx- virtual screening tool. The ligands were converted to pdb.qt file using Open Babel. Energy minimization was done using Universal force field (UFF). Figure II shows the 2D structures of the ligands imported from Pubmed.

2.5. Protein preparation

3D Crystal structure of the SGT2 (CODE: 2XQ2) protein was downloaded from RCSB, Protein Databank (PDB, http://www.pdb.org)[18] (Figure I).The protein was prepared using the protein preparation wizard of Auto dock. Water molecules present in the crystal structure were removed in the protein preparation process.[19]

2.6. Molecular Docking Studies

AutoDock Vina a molecular docking program in PyRx Virtual screening tool (0.8)[20,21] was used for the docking studies. The protein PDB file was changed into the PDBQT format. The imported ligands; tiliroside, bergenin, Swertiamarin and Bacosine were docked against the protein in a protein-ligand docking. For each ligand a docking experiment was carried out and the result was analyzed based on binding free energies and root mean square deviation (RMSD) values. The results were then ranked in the order of increasing docking energies. The lowest-energy was taken as representative binding energy of each cluster.

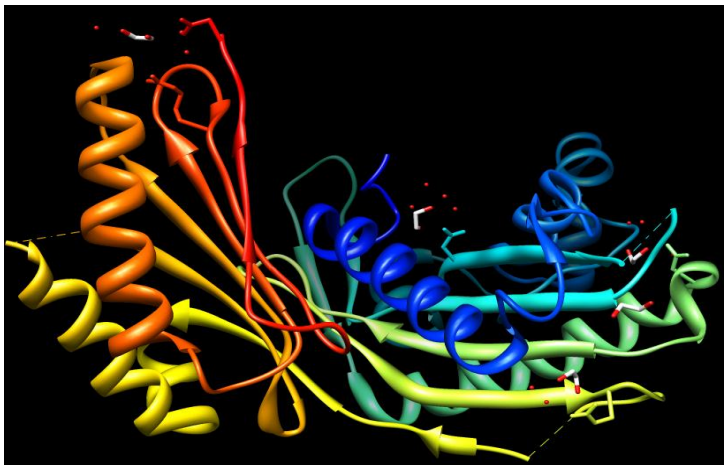
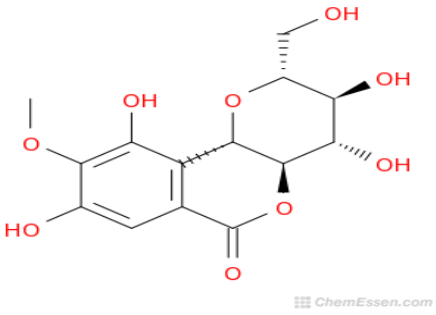
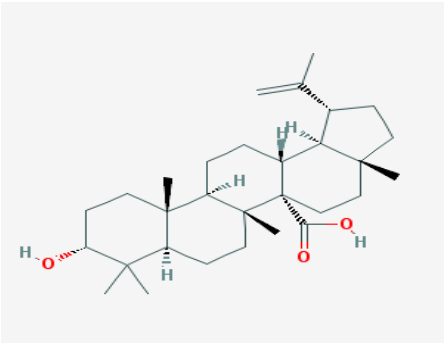


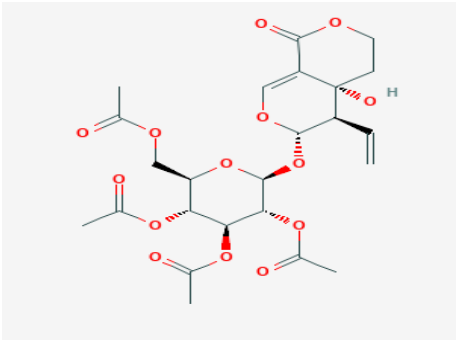
Figure I: 3D structure of SGT2 enzyme.



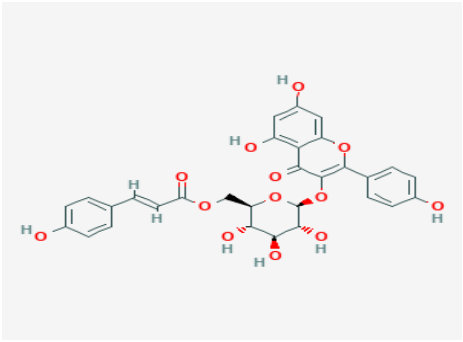
Bergenin



Bacosine



Swertiamarin



Tiliroside

3. RESULTS AND DISCUSSION

3.1. Molecular Properties of Ligands

The molecular properties of the ligands were estimated based on the Lipinski's rule of five (5) which states that an orally active drug should not violate more than one of the following rules; less than 5 hydrogen-bond donors, less than10 hydrogen-bond acceptors, a molecular mass less than 500 and log P not greater than 5.[22] The other significant properties such as total polar surface area (TPSA) and the number of rotatable bonds and molar refractivity were also calculated. TPSA of a compound should be less than 140Å2 and the number of rotatable bonds should be less than 10.[23] The results of molecular properties are shown in Table 1. Bioactivity Scores of the ligands of tiliroside, bergenin, Swertiamarin and Bacosine as GPCR ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitor (PI) and enzyme inhibitors (EI) were studied and the results were recorded as bioactivity scores. Scores greater than 0.00 suggest high activity, scores between 0.00 to -0.5 suggest mild activity and less than -0.5 suggest inactivity.[24] See Table 2.

Table 1: Molecular Properties of the ligands.

Ligands	Molecular weight	TPSA	Molar refractivity	MlogP	Rotatable bonds	H-bond donors	H-bond acceptors
Bergenin	312.27	125.68	71.63	-0.89	2	4	8
Tiliroside	594.52	216.58	149.51	-1.04	8	7	13
Swertiamarin	374.34	155.14	82.12	-2.1	4	5	10
Bacosine	455.69	60.36	134.97	5.82	2	1	3

Table 2: Bioactivity Scores of the ligands.

Ligands	GPCR	ICM	KI	NRL	PI	EI
Bergenin	0.20	0.07	0.02	0.19	0.08	0.56
Tiliroside	-0.10	-0.60	-0.24	-0.07	-0.09	0.05
Swertiamarin	0.17	0.26	-0.23	0.04	0.26	0.43
Bacosine	0.25	0.10	-0.40	0.80	0.14	0.49

Key: GPCR= G-protein coupled receptor, ICM= ion channel modulators, KI=Kinase inhibitors, NRL= nuclear receptor ligands, PI=protease inhibitors, EI= enzyme inhibitors

3.2. Pharmacokinetic Profile

Among the compounds in this study, Bacosine and bergenin showed high human intestinal absorption (HIA) while Swertiamarin and tiliroside showed low HIA. All the compounds do not permeate the blood brain barrier (BBB). The knowledge about compounds being substrate

or non-substrate of the permeability glycoprotein (P-gp) is fundamental in evaluating their active efflux through biological membranes for example from the gastrointestinal wall to the lumen.[25] The compounds in this study are not substrate of permeability glycoprotein (P-gp). The study on the potential of compounds to inhibit the cytochrome P450 (CYP) enzymes is important in determining their possible drug interactions and toxicity.[26] Approximately over 50 % of therapeutic molecules are substrate of five major isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4).[27] These enzymes are involved in metabolism of drugs.[26] It is of immense significance in drug discovery to predict the tendency of a molecule to inhibit CYPs and to determine which isoforms are affected.[15] All the compounds in this study do not inhibit any of the CYP450 enzymes except Bacosine which inhibits CYP2C9. The skin permeability (logP) measures the probability that drugs have capability to be used as transdermal. The more negative the log *K*p, the less skin permeate is the molecule.[28] All the compounds in this study are found not to be impermeable through skin Table 3.

Table 3: Pharmacokinetic Profile.

	Bacosine	**Swertiamarin**	**Tiliroside**	**Bergenin**
HIA	High	Low	Low	High
BBB permeation	No	No	No	No
P-gp substrate	No	No	No	No
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
log Kp (cm/s)	-3.12	-10	-8.17	-8.59
Bioavailability	0.56	0.11	0.17	0.55

HIA=Human intestinal absorption, BBB=blood brain barrier, P-gp= P-glycoprotein substrate

3.3. Prediction of Toxicity

Toxicity of the compounds in this study was measured in terms of toxicological endpoints, such as mutagenicity, carcinogenicity and many other endpoints. It was also measured both quantitatively in terms of LD50 (lethal dose) values, and qualitatively, such as binary (active or inactive) for certain cell types and assays or indication area such as cytotoxicity, immunotoxicity and hepatotoxicity.[29] Bacosine was predicted to be active in carcinogenicity and may have the potential to induce tumors or increase the incidence of tumors.[30] Tiliroside and bergenin show immunotoxicity and may adversely affect the immune system (Table 4). The acute toxicities of the compounds given as LD50 were also predicted using Protox-II server[17] and the toxicity classes were defined according to the globally harmonized system of classification of labeling chemicals (GHS). The results are depicted in table 5.

Table 4: Prediction of Toxicity.

Predicted Target	**Bacosine**	**Swertiamarin**	**Tiliroside**	**Bergenin**
Hepatotoxicity	Inactive	Inactive	Inactive	Inactive
Carcinogenicity	Active	Inactive	Inactive	Inactive
Immunotoxicity	Inactive	Inactive	Active	Active
Mutagenicity	Inactive	Inactive	Inactive	Inactive
Cytotoxicity	Inactive	Inactive	Inactive	Inactive

Table 5: Result of LD50.

Ligands	**LD50 (mg/kg)**	**Classification**
Bacosine	2500	Class 5
Swertiamarin	2000	Class 4
Bergenin	10000	Class 6
Tiliroside	5000	Class 5

3.4. Molecular docking Studies

The molecular docking approach is used to determine the binding affinities and energies of ligand and play a crucial role in drug discovery.[31,32] In this study, the phytochemicals Bacosine, bergenin, Swertiamarin and tiliroside were docked against sodium/glucose transporter 2. These phytochemicals have been reported to have antidiabetic activity in numerous studies but the mechanism of action is not clearly understood.[33] Ligands with lower binding energy have higher ability to bind to the receptor.[34] The results are shown in table 6. Bacosine, tiliroside and bergenin showed binding affinity comparable to the standard drug Dapagliflozin. Bacosine and tiliroside have lower binding energy (-9.1 kcal/mol and -11.0 kcal/mol respectively) than the standard drug Dapagliflozin.

Table 6: Molecular Docking Studies.

Ligand code	Ligand	Binding affinity (Kcal/mol)
71773525	Bacosine	-9.1
4098354	Swertiamarin	-8.1
71789574	Bergenin	-8.6
17654711	Tiliroside	-11.0
3819138	Dapagliflozin	-8.8

CONCLUSION

The pharmacokinetic profiles of Bacosine, bergenin, tiliroside and Swertiamarin were determined using *in silico* methods. The toxicity of the compounds was also predicted as well as their potential to inhibit the SGT2 using molecular docking studies. Among the phytochemicals in this study, Bacosine was found to have good pharmacokinetic profile and relatively low toxic potential. The docking results of bergenin also showed high binding affinity for the SGT2. These two agents may serve as potential leads for discovery of new SGT2 inhibitors. Although tiliroside showed higher affinity than the other compounds, it failed the Lipinski's rule of 5 and also showed low HIA.

Authors Declaration

The Authors declare that there is no conflict of interest.

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