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Rauwolfia serpentina in P-Glycoprotein Inhibition of Cancer & Diabetes - A Computational Study

Research Article

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Abstract

Background: The efficient treatment of cancer and diabetes is often constrained by reduced permeation of chemotherapeutic and antidiabetic medications due to p-glycoprotein-mediated efflux, impeding their therapeutic potential and necessitating higher doses or prolonged treatments, which can lead to increased toxicity and financial burden. Ayurveda, an ancient holistic healthcare system, advocates the use of herbal remedies for various ailments. Objective: This study aims to explore the potential of *Rauwolfia serpentina* and other selected antidiabetic and anticancer medicinal herbs as inhibitor of p-glycoprotein. Materials and Methods: Druggability and pharmacokinetic profile of 185 herbal constituents were evaluated and their binding affinity were interpreted against 6C0V structure of p-glycoprotein utilizing molecular docking with CDOCKER program of Discovery Studio. The stability of the docked complex was validated using molecular dynamics simulation for 40 nanoseconds. ChemMine software was employed for clustering p-glycoprotein herbal inhibitors against standard drug verapamil on the basis of physicochemical properties. Results: Docking analyses revealed that deserpidine and ajmalicine from *Rauwolfia serpentina* exhibited strong favourable binding interactions with p-glycoprotein, along with other herbal compounds viz., hydrastine from *Hydrastis canadensis*, palmatine from *Tinospora cordifolia* and hexadecanoic acid 2-hydroxy-1(hydroxymethyl)ethyl ester from *Ipomoea aquatica*. Conclusion: This study underscores the potential of combining *Rauwolfia serpentina* and other medicinal herbs with allopathic drugs to augment their bioavailability and efficacy, providing a novel avenue for advancing therapeutic strategies in oncology and diabetes management. Further experimental validation is necessary to confirm the clinical relevance of these findings.

Keywords: *Ayurveda,* Docking, Herb, Inhibitor, P-glycoprotein, *Rauwolfia serpentina.*

Introduction

Cancer, a complex and devastating disease with high mortality rate, has challenged the boundaries of medical science for decades. One of the most formidable obstacles in cancer treatment is the emergence of multidrug resistance (MDR), a phenomenon that renders chemotherapy ineffective against the relentless invasion of malignant cells (1). Diabetes mellitus (DM) is a chronic, metabolic disorder, characterized by hyperglycemia resulting from inadequate insulin secretion or resistance to insulin action, has emerged as one of the most serious disease worldwide causing life threatening complications with an estimated global prevalence of 783.2 million by 2045 (2). P-glycoprotein, an integral transmembrane protein, belonging to member of ATP binding cassette (ABC) transporter family is ubiquitously expressed in various tissues and organs involved in pharmacokinetics, such as small intestine, bile duct, kidney tubules and endothelial cells of blood–brain barrier (3). It is primarily involved in extrusion of diverse range of xenobiotics and therapeutic compounds from cells, effectively limiting their intracellular accumulation. While this mechanism is intrinsic to the body's defense against noxious substances, it often poses a significant hurdle in the context of pharmaceutical intervention, resulting in MDR. Chemotherapeutic drugs, designed to target malignant cells and antidiabetic drugs, intended to control glucose levels, frequently encounter resistance due to p-glycoprotein-mediated efflux, thereby reducing their bioavailability and effectiveness. As an adaptive response, cancer cells are known to upregulate p-glycoprotein expression to avoid chemotherapy-induced cell death (1). In diabetes, p-glycoprotein expression and activity may be altered affecting the pharmacokinetics and pharmacodynamics of orally administered medications (4). P-glycoprotein localized in the apical membrane of syncytiotrophoblast has been found to extrude glyburide, rosiglitazone, and metformin into the maternal circulation thus reducing foetal exposure (5). Furthermore, decreased p-glycoprotein expression has been seen in the blood

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vessels of diabetic patients which may contribute to the development of diabetic retinopathy (6). P-glycoprotein has been implicated in the efflux of antidiabetic drugs such as linagliptin (7), repaglinide (8) in rats thus reducing its effectiveness. Hence, substantial research has been carried out in identification of p-glycoprotein inhibitors of natural origin which when co-administered with such chemotherapeutic and antidiabetic drugs, would augment their bioavailability and circumvent drug resistance.

In Ayurveda, herbal remedies are often used to complement conventional treatments for cancer and diabetes. These herbs harbour a myriad of bioactive constituents which can alter the pharmacokinetic profile of co-administered drugs through inhibition or induction of efflux transport protein which have been recognized as the prime mechanism for herb-drug interaction (HDI) (9). Medicinally important herbs and their bioactive ingredients can lower blood sugar levels as well as control the complications of diabetes and cancer. The alkaloidal fraction of *Tinospora cordifolia* extract exerted hypoglycemic effect through improvement of insulin sensitivity and inhibition of gluconeogenesis (10). The ethanolic extract of *Tinospora plant* is also found to induce differentiation in C6 glioma cells and decrease cell proliferation through S phase and G2/M arrest with concurrent inhibition of p21 expression and cyclin-dependent kinase activity (11). Additionally, the alcoholic extract of the same plant is known to control myeloid differentiation of bone marrow progenitor cells and activate macrophages in response to tumour growth *in situ* (12). The extract of *Stevia rebaudiana* was found to be effective in reducing blood glucose level in streptozotocin-induced diabetic albino rats (13). The methanolic root extract of *Rauwolfia serpentina* demonstrated its antidiabetic effect through improvement of glycemic, antiatherogenic, and cardioprotective indices in alloxan-induced diabetic mice (14). These bioactive principles offer functional scaffolds to modulate p-glycoprotein mediated MDR (15). Majority of chemotherapeutic drugs viz., vincristine, vinblastine, etoposide, paclitaxel, topotecan, doxorubicin, daunorubicin, mitoxantrone, epirubicin (1) and antidiabetic drugs viz., glibenclamide (16); metformin (17); repaglinide (18); rosiglitazone (5); linagliptin (7); saxagliptin, vildagliptin (19); sitagliptin (20) are substrates of the efflux transporter, p-glycoprotein and therefore only partially retained by the target cell.

Here, 19 medicinal herbs with anticancer and antidiabetic proficiency viz., *Withania somnifera* (21,22), *Tinospora cordifolia* (23,22), *Caesalpinia bonducella* (24,22), *Cornus officinalis* (25,22), *Nardostachys jatamansi* (26,27), *Cyclea peltate* (28,29), *Ipomoea aquatica* (30,31), *Pausinystalia yohimbe* (32,33), *Rauwolfia serpentina* (34,12), *Digitalis purpurea* (35,36), *Stevia rebaudiana,* (37,13), *Actinodaphne hookeri* (38), *Cinchona pubescens* (39,40), *Hydrastis canadensis* (41,42), *Ricinus communis* (43,44), *Platycodon grandiflorum* (45,46), *Micromelum minutum* (47,48), *Gentiana lutea* (49,50), *Bergenia ciliata* (51,52), were chosen for the purpose of molecular docking with the efflux transporter. These herbs were selected from a variety of sources, including traditional texts viz., Ayurvedic Pharmacopoeia, ethnobotanical records and scientific studies. This selection process is guided by pharmacological data supporting specific bioactivities, alongside databases and literature on herbs and bioactive compounds.

The objective of this study is to explore the potential of *Rauwolfia serpentina* and other medicinal herbs in inhibiting p-glycoprotein, thereby improving the bioavailability and therapeutic efficacy of allopathic drugs in treating cancer and diabetes. Utilizing computational approach, we aim to identify specific compounds within these herbs that exhibit the strongest binding affinity to p-glycoprotein, offering a potential solution to drug resistance.

Materials and Methods

Preparation of protein

The three-dimensional structure of p-glycoprotein (PDB code: 6C0V) in the outward-facing conformation was retrieved from RCSB protein data bank (http://www.rcsb.org) (53). The protein was minimized utilizing Discovery Studio software which involved insertion of missing atoms, protonation of titratable residues using predicted pKs and optimization of side-chain conformations. Dogsitescorer was employed for the identification of best druggable binding pocket (https://proteins.plus/#dogsite).

Preparation of ligand

Canonical smiles of 185 ligand molecules and control drug verapamil were obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/), chEMBL (https://www.ebi.ac.uk/chembl/) and *ChemSpider* (http://www.chemspider.com/) databases. The canonical SMILES were converted into spatial data file format employing online SMILE translator (https://cactus.nci.nih.gov/translate/) and were optimized prior to docking.

Drug Likeness and ADMET analysis

Molinspiration software (http://www.molinspiration.com) was employed for evaluation of drug-likeness attributes and pkCSM tool was used to compute the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) property of the components (http://biosig.unimelb.edu.au/pkcsm/prediction).

Molecular Docking analysis

Molecular docking was carried out using CDOCKER programme of Discovery Studio which employs CHARMm (Chemistry at Harvard Macromolecular Mechanics) based docking algorithm (54). The best pose for each component was chosen based on lowest binding free energy and highest number of hydrogen and hydrophobic interactions.

Molecular Dynamics simulation

The simulation procedure can be divided into three major steps:

Input structure evaluation

The correctness of input structure is crucial in molecular dynamics simulations. A small error in the input structure can make MD simulations unreliable or lead to unrealistic trajectories. The initial structure checking page is used to check for the most common problems in MD simulation input and provide possible solutions when available. Structure checking can also be used to select fragments to be simulated if there are multiple subunits or alternate models present in the incoming structure.

The checking comprises of a list of possible alternatives to choose for the system to be simulated such as structure model, structure chain/s, residue/atom alternate locations, non-consecutive residues/sequence gaps, atom clashes including steric, alpha-carbon, polar donor, polar acceptor, apolar and ionic positive/ negative, the improper chirality, unusual peptide-bond cis configuration and disulphide bonds.

The complex that was used for the study was in sync with the major checkpoints of the evaluation scheme.

Simulation

In a discrete molecular dynamic simulation, proteins were treated as beads (Cα atoms) interacting through a discontinuous potential (55-59). Potentials were considered constant outside of discontinuities, implying that all particles behaved in a ballistic manner throughout their trajectory (constant potential, constant velocity) apart from when they reached a discontinuity. At this point, the velocities of colliding particles are adjusted by imposing conservation of linear momentum, angular momentum and total energy. As particles in this study were constrained to move within configurational space where potential energy remained constant, the kinetic energy remained unchanged and so all collisions were assumed elastic. Infinite square wells were used to describe the interaction potentials. The particle-particle distances ranged between d1 = (1 − σ)R0 and d2 = (1 + σ)R0, where R0 represents the distance in the native conformation and 2σ the width of the square well.

In line with Brownian molecular dynamics, the MD-averaged conformation was considered to be the native conformation. Only for the particles closer than a cutoff radius Rc in the native conformation, were the residue-residue interaction potentials determined. For nonconsecutive Cα particles, Rc value of 8 Å and σ value of 0.1 were employed, however for consecutive pairs of residues, a smaller well width (σ = 0.05) was chosen to keep the Cα-Cα distances closer to the anticipated value of 3.8 Å.

This description of the potential accurately reproduced the shape of the wells as obtained by the distance-dependent pseudoharmonic model (60).

Trajectory analyses

GROMAC's commands were used to run trajectory analyses:

- G_rms from the Gromacs package (XTC trajectory format) for calculation of Root Mean Square Deviation (RMSD) along the trajectory.
- G_rmsf, a Gromacs package function for calculation of the average Root Mean Square deviation for each residue along the trajectory (XTC trajectory format).
- G_rmsf from the Gromacs package (XTC trajectory format) for calculation of B Factor values for each residue.

Clustering analysis

ChemMine web tool was utilised for clustering small molecules based on structural similarities and physicochemical descriptors (61). The JOELib tool was employed for estimation of 38 physicochemical descriptors for each small molecule (https://chemminetools.ucr.edu/tools/launch_job/Properties/).

Results

A comprehensive computational analysis was conducted to explore the potential of *Rauwolfia serpentina* and other medicinal herbs in p-glycoprotein inhibition.

Drug likeness and ADMET analysis

185 components of 19 anticancer and antidiabetic herbs were initially shortlisted based on drug likeness and pharmacokinetic attributes. The analysis showed that 179 components adhered to Lipinski's rule of five (62), indicating good drug likeness properties. Pharmacokinetic study revealed that 30 components serve as p-glycoprotein inhibitors.

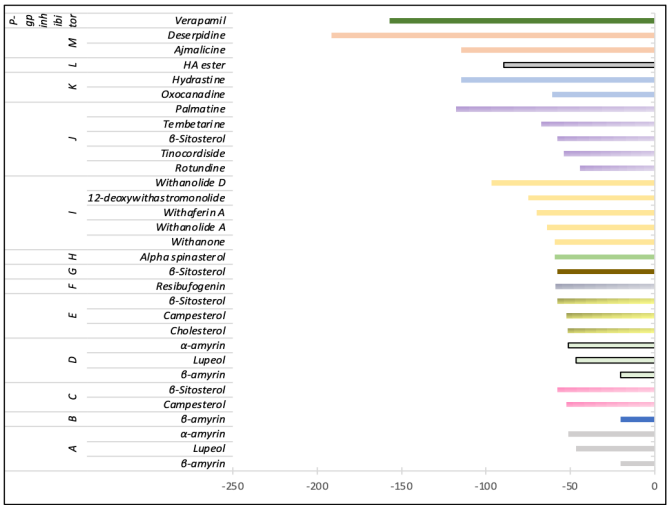
Molecular docking analysis

To interpret the binding affinity of phytoconstituents with p-glycoprotein, molecular docking was performed using Discovery Studio. The p-glycoprotein herbal inhibitors were chronologically analysed on basis of minimal binding energy viz., deserpidine, palmatine, ajmalicine, hydrastine, withanolide D, hexadecanoic acid 2-hydroxy-1 (hydroxymethyl) ethyl ester (HA ester), 12-deoxywithastromonolide, withaferin A, tembetarine, withanolide A, oxocanadine, alpha spinasterol, withanone, resibufogenin, β-sitosterol, tinocordiside, campesterol, cholesterol, α-amyrin, lupeol, rotundine and β-amyrin. Binding energy of interaction was found to be minimum in case of deserpidine (-191.47 Kcal/mol), a component present in *Rauwolfia serpentina* (Figure 1).

In order to evaluate the specificity of protein-ligand interactions and prediction of appropriate binding pose, the hydrogen bonds and hydrophobic interactions were calculated. The p-glycoprotein herbal inhibitors were chronologically analysed on basis of maximum hydrogen bond interactions viz., deserpidine, hydrastine, withanolide D, withaferin A, hexadecanoic acid 2-hydroxy-1(hydroxymethyl)ethyl ester (HA ester),

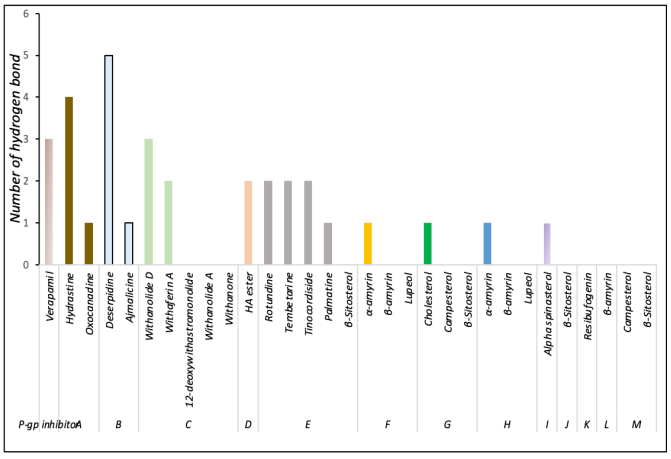
rotundine, tembetarine, tinocordiside, oxocanadine, ajmalicine, palmatine, α-amyrin, cholesterol and alpha spinasterol. Highest number of hydrogen bonds was found to be 05 in deserpidine from *Rauwolfia serpentina* (Figure 2).

Figure 1: Binding energy of interaction of p-glycoprotein inhibitors in anticancer and antidiabetic herbs



(A) Caesalpinia bonducella; (B) Gentiana lutea; (C) Stevia rebaudiana; (D) Ricinus communis; (E) Digitalis purpurea; (F) Nardostachys jatamansi; (G) Actinodaphne hookeri; (H) Platycodon grandiflorum; (I) Withania somnifera; (J) Tinospora cordifolia; (K) Hydrastis canadensis; (L) Ipomoea aquatica; (M) Rauwolfia serpentina. Verapamil is considered as the control.

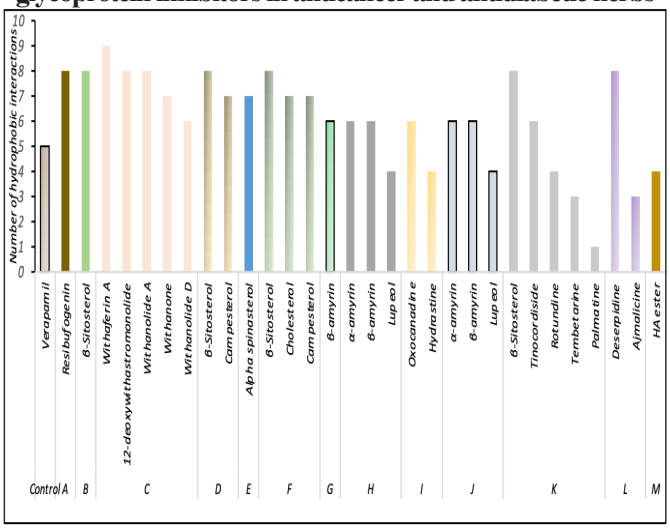
Figure 2: Number of hydrogen bonds of p-glycoprotein inhibitors in anticancer and antidiabetic herbs



(A) Hydrastis canadensis; (B) Rauwolfia serpentina; (C) Withania somnifera; (D) Ipomoea aquatica; (E) Tinospora cordifolia; (F) Caesalpinia bonducella; (G) Digitalis purpurea; (H) Ricinus communis; (I) Platycodon grandiflorum; (J) Actinodaphne hookeri; (K) Nardostachys jatamansi; (L) Gentiana lutea; (M) Stevia rebaudiana. Verapamil is considered as the control.

The p-glycoprotein herbal inhibitors were sequentially analysed on the basis of maximum hydrophobic interactions viz., withaferin A, deserpidine, resibufogenin, β-sitosterol, withanolide A, 12-deoxywithastromonolide, withanone, alpha spinasterol, campesterol, cholesterol, withanolide D, α-amyrin, β-amyrin, oxocanadine, tinocordiside, lupeol, hydrastine, rotundine, hexadecanoic acid 2-hydroxy-1 (hydroxymethyl)ethyl ester (HA ester), tembetarine, ajmalicine and palmatine. Maximal hydrophobic interactions were observed to be 09 in withaferin A from *Withania somnifera*. Deserpidine, a component from *Rauwolfia serpentina* exhibited 8 hydrophobic interactions (Figure 3).

Figure 3: Number of hydrophobic interactions of p-glycoprotein inhibitors in anticancer and antidiabetic herbs



(A) Nardostachys jatamansi; (B) Actinodaphne hookeri; (C) Withania somnifera; (D) Stevia rebaudiana; (E) Digitalis purpurea; (F) Platycodon grandiflorum; (G) Gentiana lutea; (H) Caesalpinia bonducella; (I) Hydrastis canadensis; (J) Ricinus communis; (K) Tinospora cordifolia; (L) Rauwolfia serpentina; (M) Ipomoea aquatica. Verapamil is considered as the control.

In this study, a library of 185 phytoconstituents derived from medicinal herbs were screened and 179 components were docked into the best druggable binding pocket of p-glycoprotein. The docking results indicated that the phytoconstituents viz., deserpidine, hydrastine and ajmalicine exhibit strong binding interactions with the efflux transporter. The two-dimensional structures, binding energy and details of various interactions of the top three components are displayed in Table 1. Deserpidine was found to have a higher binding energy of -191.47 Kcal/mol than the positive control verapamil (-157.24 Kcal/mol). Deserpidine was engaged in five hydrogen bond interactions with LEU332, GLU972, LEU975, THR76, SER979 and eight hydrophobic interactions with PHE72, PHE79, PHE732, LEU332, LEU975, LEU976, ILE736, ILE328. On the contrary, hydrastine showed four hydrogen bonds with residues GLU972, LEU976, THR76, SER979 and four hydrophobic interactions with LEU332, LEU976, ILE736, PHE72. Ajmalicine was involved in one hydrogen bond with amino acid residue GLU972 and three hydrophobic interactions with residues LEU332, LEU976 and ILE736 (Figure 4). Deserpidine was found to be the best compound among 185 components on account of minimal binding free energy and maximum number of hydrogen and hydrophobic interactions.

Table 1: List of top three herbal phytoconstituents selected based on lowest binding energy, number of hydrogen and hydrophobic interactions.

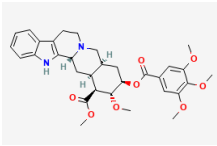
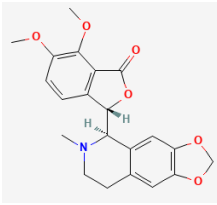
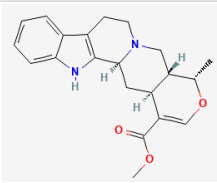
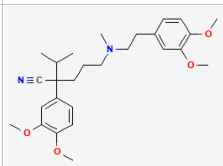
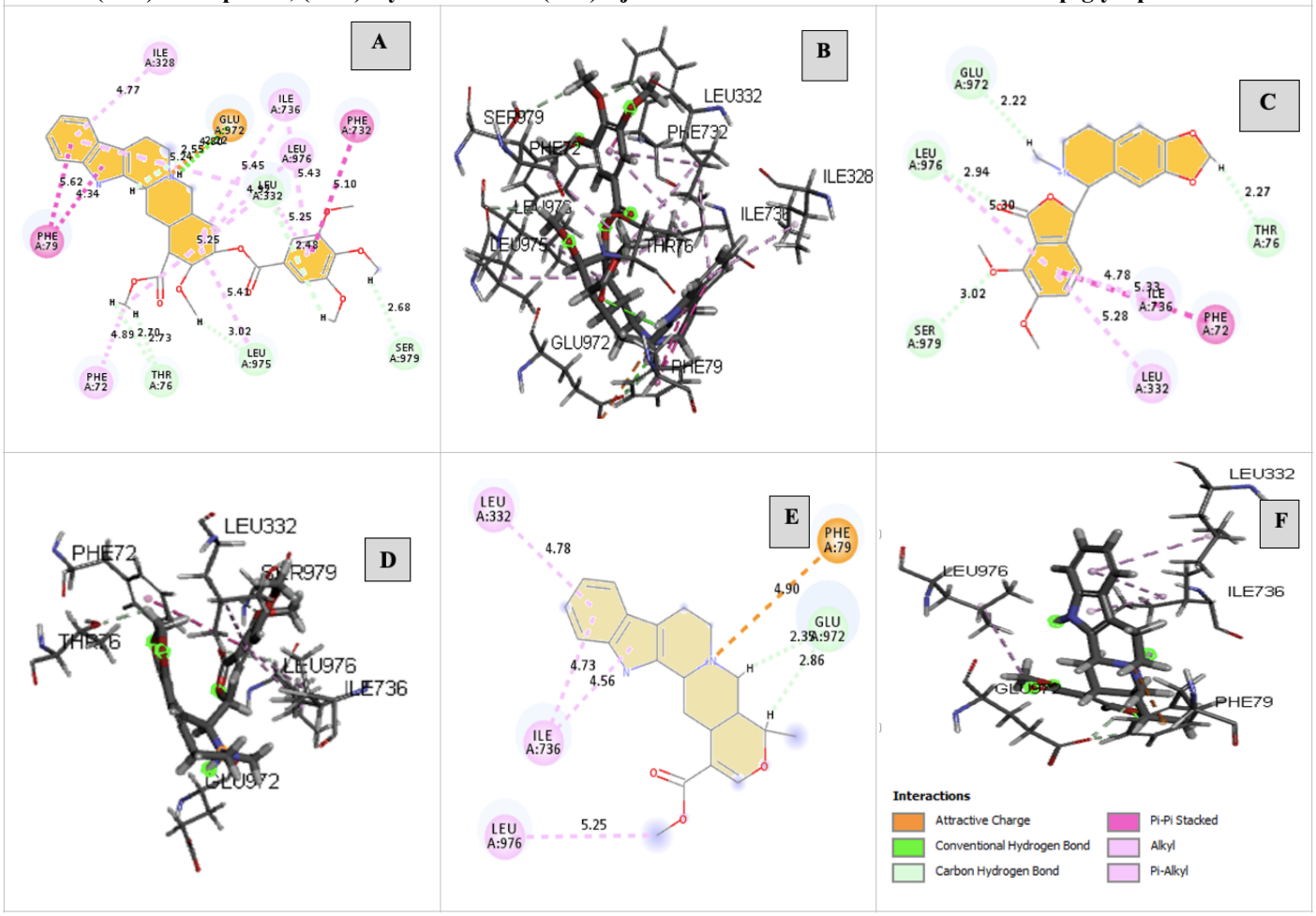
S.No.	Phytoconstituent	2D structure	Binding energy (Kcal/mol)	Molecular interactions
1	Deserpidine (CID_8550)		-191.47	Hydrogen bond: LEU332 (5.25 Å), GLU972 (5.24 Å), LEU975 (3.02 Å), THR76 (4.89 Å), SER979 (2.68 Å) Pi-pi stacked: PHE732 (5.10 Å), PHE79 (5.62 Å) Pi-alkyl: PHE72 (4.89 Å), LEU976 (5.43 Å), LEU975 (5.43 Å) Alkyl: ILE328 (4.77 Å), ILE736 (5.45 Å), LEU332 (5.41 Å)
2	Hydrastine (CID_197835)		-114.69	Hydrogen bond: GLU972 (2.22 Å), LEU976 (2.94 Å), THR76 (2.27 Å), SER979 (3.02 Å) Pi-pi T-shaped: PHE72 (5.33 Å) Pi-alkyl: LEU332 (5.28 Å), LEU976 (5.30 Å), ILE736 (4.78 Å)
3	Ajmalicine (CID_441975)		-114.79	Hydrogen bond: GLU972 (2.86 Å) Pi-alkyl: LEU976 (5.25 Å) Alkyl: LEU332 (4.78 Å), ILE736 (4.73 Å)
4	Verapamil (CID_2520)		-157.24	Hydrogen bond: LEU332 (2.36 Å), LEU975 (2.94 Å), ILE736 (2.59 Å) Pi-alkyl: ALA729 (4.90 Å), PHE79 (4.59 Å), ILE736 (5.17 Å) Pi-pi stacked: PHE336 (5.63 Å) Pi-pi T-shaped: PHE732 (4.46 Å)

Figure 4: 2D (A,C,E) and 3D (B,D,F) diagrams show the interactions and preferred binding poses of inhibitors: (A-B) Deserpidine, (C-D) Hydrastine and (E-F) Ajmalicine with 6C0V structure of human p-glycoprotein.



Molecular dynamics simulation

Molecular dynamics simulation was performed using GROMACS software for a period of 40 nanoseconds to assess the stability of p-glycoprotein-deserpidine complex under dynamic conditions. The best pose was obtained from the molecular docking experiment by CDOCKER and subjected to MD simulation.

RMSD is an important parameter which measures the deviation of the positions of atoms or residues in a molecular system, from their initial positions over the course of a simulation. This information is crucial for understanding the structural dynamics, interactions and stability of biomolecules. In this study, the RMSD value per residue was analysed to assess the stability of the docked complex. When the protein was bound with the ligand, uniformity was observed in RMSD fluctuation in the context of amino acid residues (Figure 5).

Another key criterion for determining the stability of the complex is the B factor, also known as the temperature factor or Debye-Waller factor. It indicates the average squared displacement of every atom over time from its mean position. Analysing B factor can reveal the movement of different regions of a molecule and fluctuation over time. The uniformity in B factor graph is a sign that the complex has remained stable during the entire duration of simulation (Figure 6). Therefore, the interaction of deserpidine with p-glycoprotein is stable and the desired effect of the small molecule should be imparted.

Figure 5: Root Mean Square Deviation curve of p-glycoprotein-deserpidine complex.

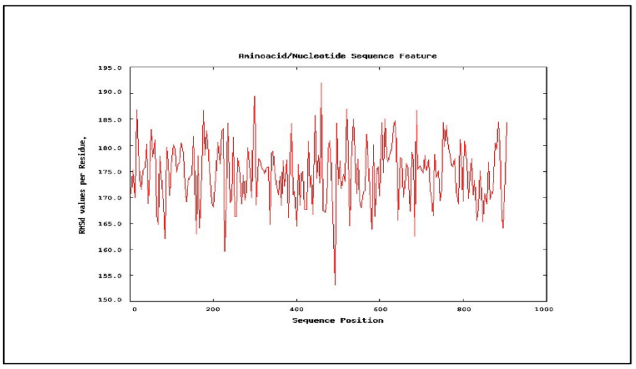
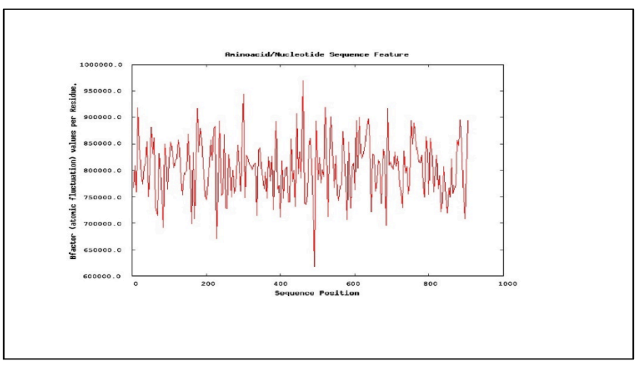


Figure 6: B factor graph of p-glycoprotein-deserpidine complex.

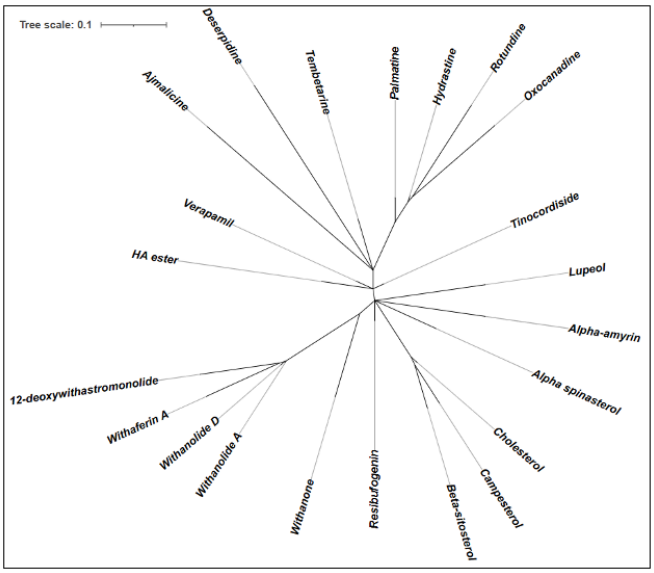


With a box size of 10, the TIPS3BOX solvent model was successfully implemented for the evaluation of the p-glycoprotein-deserpidine complex. This resulted in a stable B factor and RMSD, indicating that simulation was successful and the complex was stable, showing that the ligand was binding efficiently with the receptor.

Clustering analysis

Clustering of small molecules based on structural and physicochemical similarities is a strategy for relating their structural features with functions. The clustering tree is depicted in Figure 7. Deserpidine is clustering with a known inhibitor verapamil (control) and other eight compounds such as hydrastine, ajmalicine, 2-hydroxy-1(hydroxymethyl)ethyl ester (HA ester), rotundine, tembetarine, tinocordiside, oxocanadine, palmatine, which are p-glycoprotein inhibitors. Deserpidine shows similarity to this group of small molecules according to physicochemical properties.

Figure 7: Clustering of p-glycoprotein inhibitors based on structural similarity and physicochemical attributes computed by ChemMine software



Discussion

With the rapid rise of diabetes incidence, there is an urgent need for safe and effective herbal biologically active ingredients with antidiabetic potential. Chemotherapeutic drugs, often plagued by multidrug resistance, could potentially be administered in combination with herbal p-glycoprotein inhibitors, significantly elevating their therapeutic potential. This may not only potentiate the drug's bioavailability but also curtail the adverse effects associated with chemotherapy, thereby improving the overall quality of life for cancer patients. Chemotherapeutic and antidiabetic drugs are effluxed out from the target cell membrane due to altered expression of p-glycoprotein. Inhibiting the transport function of p-glycoprotein would increase the concentration of the concerned drugs as there would be limited drug outflow from the target cell membrane.

The efficacy of herbal phytoconstituents in modulation of p-glycoprotein mediated efflux have been observed in case of reserpine (*Rauwolfia serpentina*) (63); p-glycoprotein inhibitory activity by kaempferol in Caco-2 cells and multidrug-resistant 1 transfected MDCK cells (*Stevia rebaudiana*) (64); reversal of MDR in KB CH^R 8-5 cells by quercetin (*Cornus officinalis*) (65). These reflect the role of herbal components in inhibiting p-glycoprotein mediated drug efflux which allows retention of drugs inside the target cell.

Medicinal herbs with its wide spectrum of active ingredients have shown therapeutic effect in various animal models and in cancer and diabetic patients. These phytoconstituents can act on multiple molecular targets and several mechanisms may be involved in glucose-lowering and/or anticancer action, including: regulation of signalling pathways, accelerated GLUT4 translocation, lowering of oxidative stress, suppression of pro-inflammatory cytokines production, upregulation of PPARγ gene expression, inhibition of angiogenesis, anti-inflammatory effect and immunomodulatory action (66,67). Withaferin A, a withanolide from *Withania somnifera* was found to increase glucose uptake in skeletal myotubes (68) as well as inhibit inflammatory response in pancreatic beta cells and protect against cytokine induced damage in mice and human islets (69,70). It has also been extensively studied for its anticancer effects and numerous interactions contributing to its anticancer effect have been investigated. When human cervical cancer cells were treated with withaferin A, the expression of human papillomavirus oncoproteins was lowered, p53 was induced and cell proliferation was suppressed. Human endometrial cancer cell proliferation was reported to be inhibited by withaferin A through modulation of TGF-β signaling and by preventing TGF-β dependent Smad2 phosphorylation (71). Hydrastine, an isoquinoline alkaloid from *Hydrastis canadensis* exhibited anticancer effect through suppression of invasion of human lung adenocarcinoma cells along with inhibition of the PAK4/LIMK1/cofilin, PAK4/SCG10 and PAK4/MMP2 pathways (72). Stevioside, a glycoside from *Stevia rebaudiana* was shown to increase insulin level and curtail gluconeogenesis through downregulation of phosphoenolpyruvate carboxykinase gene in rat liver (73). In addition, it reduced fasting blood glucose level and glycosylated haemoglobin amount in streptozotocin- induced diabetic rats (13). Kaempferol from *Stevia rebaudiana* was reported to inhibit NF-κB pathway activation which led to amelioration of defect in insulin signalling pathway (74). Moreover, it exerted protective effect on pancreatic β-cell through improved cAMP signalling and inhibition of apoptosis (75) as well as lowered fasting blood glucose level (76). Quercetin, one of the most distributed flavonol from *Cornus officinalis* exhibit its antidiabetic effect through protection against streptozotocin-induced β-cell damage in rat pancreas (77), inhibition of glucose transporter GLUT2 (78) and also α-amylase, α-glucosidase (79). Moreover, it exerted anticancer effect through down regulation of the expression of IQGAP1 and ERK and suppression of STAT3 and PI3K/AKT/mTOR pathways in primary effusion lymphoma cells (80). Therefore, concomitant administration of these valuable phytochemicals would increase the serum concentration of the desired drugs inside the cell as well as impart additional therapeutic effect.

The herbal phytoconstituents with significant p-glycoprotein inhibitory effect may be analysed based on their magnitude of negative binding free energy and number of hydrogen bonds and hydrophobic interactions. These are vital parameters for determining binding affinity in protein-ligand interactions as they contribute to the stability and specificity of the complex. The strength of these interactions can provide insights into the thermodynamics and kinetics of the binding process and potentially recognise ways to optimise ligand binding (81).

Verapamil is an established and extensively studied inhibitor of p-glycoprotein (82) with a well-defined interaction profile. Its effect on p-glycoprotein makes it a reliable standard for evaluating the pharmacokinetic implications of p-glycoprotein modulation. Its role as a calcium channel blocker, along with its vasodilatory and cardiac effects, allows for the examination of p-glycoprotein inhibition in the context of various physiological pathways, providing insights into drug behaviour that may not be fully captured by antidiabetic or anticancer drugs. Deserpidine exhibited binding energy of -191.47 Kcal/mol which was better than the standard drug verapamil (-157.24 Kcal/mol). Verapamil was engaged in three hydrogen bond interactions (carbon-hydrogen) with LEU332 (2.36 Å), LEU975 (2.94 Å), ILE736 (2.59 Å) and five hydrophobic (one pi-pi T-shaped, one pi-pi stacked and three pi-alkyl) interactions with residues PHE732 (4.46 Å), PHE336 (5.63 Å), PHE79 (4.59 Å), ALA729 (4.90 Å), ILE736 (5.17Å) respectively whereas deserpidine showed better binding affinity with five hydrogen bond interactions and eight hydrophobic interactions at the same binding site. Thus, deserpidine, a non-toxic bioactive ingredient of *Rauwolfia serpentina* with its stronger and favorable binding interactions than verapamil maybe considered as the lead compound in the circumvention of drug efflux mediated by p-glycoprotein. Combining herbal phytoconstituents with conventional drugs could enhance their therapeutic effects by increasing intracellular drug concentrations.

Conclusion

The natural compounds enumerated in this study have the potential to inhibit p-glycoprotein, displaying promising druggability and ADMET attributes, allowing retention of substrate drugs, thereby making them potential lead molecules for co-administration. Cluster analysis by physicochemical similarities further validated that deserpidine belongs to the same group of compounds which are known inhibitors of p-glycoprotein. Therefore, deserpidine from *Rauwolfia serpentina* is identified as the lead inhibitory molecule, followed by hydrastine and ajmalicine, based on analysis of a pool of 19 herbs with anticancer and antidiabetic properties. These compounds may play

crucial role in evasion of drug resistance conferred by p-glycoprotein as well as augment therapeutic action. Further *in vitro* and *in vivo* studies are warranted to validate their efficacy as adjuncts in the treatment of cancer and diabetes.

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Conflict of Interest

There is no conflict of interest.

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