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RESEARCH ARTICLE

Exploring the Potential of Phytoconstituents for Immunomodulation as Natural Inhibitors of P-glycoprotein - A Computational Study

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Abstract

Background: P-glycoprotein is an efflux transporter found on the walls of internal organs that interact with the environment, to reduce bioavailability of xenobiotics and chemotherapeutic drugs. Co-administration of herbal medicinal components as modulators or inhibitors may configure the transporter in favour of the drug, thus restricting its expulsion. **Aim:** The present study aims to explore such herbal medicinal components as potential p-glycoprotein inhibitors thus analyzing the possibility for retaining co-administered immunomodulatory medicines within target cells. **Methods:** Druggability and pharmacokinetic attributes of the natural compounds were analyzed. Molecular docking using CDOCKER program of Discovery studio (version 2019-21) was utilized to analyze the binding affinity between the natural compounds and 6C0V receptor. Molecular dynamics simulation was employed to evaluate the stability of potential binding compounds. **Results:** Molecular docking analyses of 462 components from 30 herbs revealed that the inhibitors *viz*., chelidonine, dihydroberberine, stylopine, fumariline from *Chelidonium majus*; akuammigine, echitamine from *Alstonia scholaris*; arjunone from *Terminalia arjuna* and pareirubrine A from *Cissampelos pareira* displayed strong binding affinity towards p-glycoprotein. **Conclusion:** These lead compounds *viz.*, chelidonine, akuammigine and echitamine bearing the maximum binding potency, are known components of immune enhancing herbs. We propose that these molecules may be used in conjunction with immunomodulatory drugs to enhance their bioavailability.

Keywords : Docking, Immunomodulatory, Inhibitors, Medicinal Herbs, P-glycoprotein

Abbreviations: MDR: Multidrug Resistance; (ABC) transporter: ATP-Binding Cassette Transporter; ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; MD Simulation: Molecular Dynamics Simulation; RMSD: Root Mean Square Deviation; BBB: Blood-Brain Barrier

1. Introduction

Multi Drug Resistance (MDR) is considered as one of the major impediments in successful treatment of several therapeutic drugs. One of the mechanisms underlying MDR involves the expulsion of drugs from cells over expressing p-glycoprotein, an ATP-Binding Cassette (ABC) transporter. Human p-glycoprotein is present in cells of the blood brain barrier, kidney tubules, small intestine, heart, lungs, and on the surface of immune cells, viz., T-cells, B-cells, macrophages, *etc*. where it functions as a physiological barrier by pumping toxins and xenobiotics out of cells[1]. P-glycoprotein is also known for its immunomodulatory effects, influencing the secretion of various inflammatory mediators, including prostaglandins, platelet-activating factor and cytokines[1]. Inhibiting the transport function of p-glycoprotein can result in significant clinical drug interactions and enhance the bioavailability of allopathic drugs within the target cell. Medicinal herbs have been utilized in treatment of several diseases and are regarded

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as inexhaustible sources of novel pharmacologically bioactive compounds. Herbal components possess functional scaffolds to alter transport function of p-glycoprotein and reverse multidrug resistance[2,3]. The major therapeutic immune modulatory drugs *viz.*, prednisone[4], tacrolimus[5], cyclosporine and sirolimus[6], dexamethasone[7], pomalidomide[8], levamisole[9] and tofacitinib[10] act as substrates for p-glycoprotein and are consequently expelled upon administration.

So, the active components of thirty herbs with immune stimulatory proficiency, *viz.*, *Boerhavia diffusa, Psoralea corylifolia, Commiphora wightii, Terminalia chebula, Aloe barbadensis, Vitex negundo, Stellera chamaejasme, Nelumbo nucifera, Boswellia serrata, Swertia chirata, Mucuna pruriens, Galanthus nivalis, Chelidonium majus, Bacopa monnieri*, *Eclipta alba, Semecarpus anacardium, Ficus religiosa, Alstonia scholaris, Hemidesmus indicus, Pterocarpus marsupium, Salicornia herbacea, Terminalia arjuna, Centella asiatica, Ficus racemosa, Nerium odorum, Saraca asoca, Abutilon indicum, Plumbago zeylanica, Cissampelos pareira* and *Catharanthus roseus*[11-26] have been selected for assessing their inhibitory binding potency with the efflux transporter through molecular docking. Known immune modulatory herbs have been selected to identify immune modulator phytoconstituents with potential to interact with the efflux transporter through molecular docking. By assessing the binding potency of these components with the efflux transporter, the study aims to uncover specific interactions that could provide insights into their mechanisms of immune modulation and identify lead compounds that may enhance immune response *via* transporter modulation. This study would help identify potential medicinal herbs as inhibitors of p-glycoprotein, thus increasing the accumulation of concerned drugs inside the cell.

2. Materials and Methods

2.1 Protein Preparation

To study the molecular docking between p-glycoprotein and herbal components, p-glycoprotein structure (PDB code: 6C0V)[27] was used. The outward-facing conformation of p-glycoprotein was retrieved from the protein data bank website[27]. Dogsite scorer server[28] was employed to detect the binding pockets of p-glycoprotein.

Utilizing "prepare protein" protocol in Discovery studio, various tasks were executed, including the insertion of missing atoms, standardization of atom names and protonation of titratable residues through predicted pKs.

2.2 Ligand Preparation

Molecular docking preparations involved obtaining the canonical smiles of 462 bioactive molecules, as well as controls (verapamil, doxycycline and elacridar), from the PubChem and Chembl databases. The conversion of canonical SMILES to SDF files was accomplished through online SMILE translator[29]. The ligands were optimized using prepare or filter ligands protocol in Discovery studio to assign proper bond orders and generate accessible tautomers, stereoisomers and ionization states.

2.3 Druggability and Pharmacokinetic Study

The Molinspiration tool was employed for assessing the druggability of the molecules, while a pharmacokinetic study was conducted to examine the ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) attributes of the components.

2.4 Molecular Docking Analysis

The protein-ligand docking investigation was conducted using the CDOCKER program of Discovery studio. CDOCKER employs a grid-based molecular docking approach, utilizing the CHARMm-based molecular dynamics scheme to facilitate the docking of ligands into binding site of a receptor[29].

2.5 Molecular Dynamics Simulation

Discrete Molecular Dynamics (MD) simulation was performed where the protein was treated as a system of beads representing Cα atoms, which interacted through a discontinuous potential[30-37]. Beyond these discontinuities, the potentials were assumed to remain constant, resulting in a ballistic regime for the particles, where their velocity remained constant under all conditions, except when the particles encountered a potential discontinuity.

Infinite square wells were utilized to represent the interaction potentials. The distances between particles spanned from d1 = (1 − σ)R0 to d2 = (1 + σ)R0, where R0 denoted the distance in the native conformation, and σ indicated the width of the square well. Following the principles of Brownian molecular dynamics, the MD-averaged conformation was identified as the native conformation and residue-residue interaction potentials were determined solely for particles within a cutoff radius, Rc. Nonconsecutive Cα particles used a Rc of 8 Å and a σ value of 0.1, while consecutive pairs of residues employed a narrower well width (σ = 0.05) to maintain Cα-Cα distances close to the anticipated 3.8 Å. This potential definition closely matched the well shape derived from Kovacs *et al* distance-dependent pseudo harmonic model[38].

Trajectory analysis was conducted by executing GROMACS commands, which included the assessment of Root Mean Square Deviation (RMSD) along the trajectory using the 'g_rms' tool from the GROMACS package in XTC trajectory format. Additionally, the average root mean square deviation per residue along the trajectory was determined with the 'g_rmsf' tool. Further more, B factor values per residue were obtained from the trajectory using the 'g_rmsf' tool from the GROMACS package and XTC trajectory format.

3. Result

The components of thirty immunomodulatory herbs were subjected to screening based on druggability and pharmacokinetic properties. The drug-likeness score of the components were computed using Lipinski's rule of five, with a cutoff of >40%. Out of the screened compounds, 379 exhibited drug-likeness properties, while 83 compounds did not meet rule of five criteria. ADMET study was conducted using pkCSM server. 93 components were found to be p-glycoprotein inhibitors, from a pool of 379 compounds and were therefore evaluated for their binding potency.

3.1 Drug-Likeness

The drug-likeness attributes of 30 components have been enlisted in Table 1. The compounds exhibit acceptable drug-like characteristics. According to Lipinski's rule of five, the active drug molecule should not exceed one violation in parameters, including molecular weight ≤ 500, LogP ≤ 5 and the number of hydrogen bond acceptors and donors both being ≤ 10 and ≤ 5, respectively. Values of hydrogen bond acceptors and donors, molecular weight suggest that small molecules have good membrane permeability and oral bioavailability. The octanol-water partition coefficient (LogP) serves as a valuable metric in rational drug design, assessing molecular hydrophobicity, a crucial factor influencing drug bioavailability, drug-receptor interactions, metabolism and toxicity.

3.2 ADMET Analysis

The ADMET features were predicted using pkCSM which relies on graph-based signatures and revealed that 93 components serve as p-glycoprotein inhibitors. ADMET characteristics impact the bioavailability and metabolism of bioactive molecules and therefore their calculation is imperative towards optimizing them.

The result of pharmacokinetic study of 30 components have been summarized in Table 2. The ADMET values of the compounds were found to be within an acceptable range. A component with intestinal absorption of less than 30% in the body is considered as poorly absorbed. In this study, all the compounds showed good intestinal absorption. Blood-Brain (BB) permeability, assessed by the logarithmic ratio of drug concentration in the brain to that in the plasma, is an important parameter to consider while improving the efficiency of drugs which are active within the brain. A compound with log BB> 0.3 is considered to easily penetrate the Blood-Brain Barrier (BBB) whereas, log BB<-1 indicates lower BBB penetration. The majority of the compounds exhibited moderate BBB penetration and were not inhibitors of cytochrome CYP2D6. CYP2D6 plays a significant role in the metabolism of a diverse array of substrates in the liver and its inhibition by a drug accounts for the majority of drug-drug interaction cases. From the prediction of water solubility which is expressed as the logarithm of the molar concentration, most of the compounds were found to be water soluble. The efficiency of a particular drug can be influenced by the degree to which it binds proteins within blood, as the bound fraction is temporarily shielded from metabolism and the pharmacological effect is exhibited by only the unbound fraction.

Table 1. Drug-likeness attributes of natural inhibitors

S. No.	Components	Molecular Weight	Log P	No. of H Bond Donors	No. of H Bond Acceptors	No. of Violations
1	Chelidonine	353.37	2.19	1	6	0
2	Akuammigine	352.43	3.41	1	5	0
3	Echitamine	385.48	-2.27	3	6	0
4	Dihydroberberine	337.38	3.10	0	5	0
5	Stylopine	323.35	3.04	0	5	0
6	Arjunone	351.36	2.86	0	6	0
7	Pareirubrine A	367.36	2.49	1	7	0
8	Fumariline	351.36	2.86	0	6	0
9	Corylifol A	390.48	6.66	2	4	1
10	Corylifolinin	324.38	4.81	3	4	0
11	Syringaresinol	418.44	2.62	2	8	0
12	Akuammicine-N-Oxide	338.41	3.38	1	5	0
13	Tocopherol	416.69	8.98	1	2	1
14	Myrrhanol	460.74	7.69	3	3	1
15	Isofucosterol	412.70	7.69	1	1	1
16	Sanguinarine	332.33	0.80	0	5	0
17	9,10-Di(methylthio)hexadecanoic acid, methyl ester	362.64	6.91	0	2	1
18	Di-N-Decylsulfone	346.62	8.50	0	2	1
19	16-Dehydropregnenolone	314.47	3.81	1	2	0
20	Stigmasterol	412.70	7.87	1	1	1
21	Digitoxigenin	374.52	2.47	2	4	0
22	Bavachin	324.38	4.45	2	4	0
23	Dasycarpidan-1-methanol, acetate	326.44	3.97	1	4	0
24	Methyl oleanolate	470.74	7.34	1	3	1
25	Swertanone	424.71	8.07	0	1	1
26.	Elliptinone	374.35	3.31	2	6	0
27	Corialstonine	410.47	3.10	0	7	0
28	β-Sitosterol	414.72	8.62	1	1	1
29	α-Amyrin acetate	468.77	8.58	0	2	1
30	Lanosterol	426.73	8.48	1	1	1

3.3 Molecular Docking Analysis

To gain a deeper understanding of the binding interactions between natural components and p-glycoprotein, molecular docking was performed utilizing Discovery studio. Verapamil, doxycycline and elacridar were chosen as reference drugs.

Figure 1 depicts the binding energy of p-glycoprotein inhibitors in immunomodulatory herbs *viz*., **(A)** *E. alba*; **(B)** *A. barbadensis*; **(C)** *B. diffusa*; **(D)** *N. odorum*; **(E)** *B. serrata*; **(F)** *P. zeylanica*; **(G)** *F. racemosa*; **(H)** *P. marsupium*; **(I)** *H. indicus*; **(J)** *F. religiosa;* **(K)** *A. indicum*; **(L)** *S. chirata*; **(M)** *C. roseus*; **(N)** *C. wightii*; **(O)** *P. corylifolia*; **(P)** *V. negundo*; **(Q)** *C. pareira*; **(R)** *T. arjuna*; **(S)** *A. scholaris and* **(T)** *C. majus* (in sequence of their efficiency) where verapamil, doxycycline and elacridar are considered as the control.

Table 2. ADMET attributes of natural inhibitors

S. No.	Components	Water Solubility (log mol/L)	Cytochrome P450 2D6 Inhibition	Intestinal Absorption (% absorbed)	BBB Permeability (log BB)	Fraction Unbound
1	Chelidonine	3.927	No	95.957	-0.332	0.122
2	Akuammigine	-3.824	Yes	94.29	0.51	0.228
3	Echitamine	-2.584	No	98.138	-0.212	0.307
4	Dihydroberberine	-4.211	No	95.29	0.099	0.026
5	Stylopine	-3.699	Yes	96.054	0.186	0.138
6	Arjunone	-5.043	No	96.679	-0.3	0.131
7	Pareirubrine A	-3.361	No	98.422	-0.82	0.149
8	Fumariline	-3.574	No	98.647	-0.015	0.186
9	Corylifol A	-5.036	No	93.752	-0.141	0
10	Corylifolinin	-3.532	No	90.912	-0.818	0.08
11	Syringaresinol	-3.92	No	78.823	-0.771	0
12	Akuammicine-N-Oxide	-4.807	No	97.691	0.034	0.095
13	Tocopherol	-7.417	No	89.452	0.915	0
14	Myrrhanol	-6.623	No	89.76	-0.517	0
15	Isofucosterol	-6.715	No	94.642	0.764	0
16	Sanguinarine	-5.56	Yes	100	-0.105	0.265
17	9,10-Di(methylthio) hexadecanoic acid, methyl ester	-7.253	No	91.36	0.776	0.035
18	Di-N-Decylsulfone	-6.064	No	91.975	-0.239	0
19	16-Dehydropregnenolone	-4.735	No	96.151	0.148	0.123
20	Stigmasterol	-6.682	No	94.97	0.771	0
21	Digitoxigenin	-4.915	No	96.564	-0.018	0.185
22	Bavachin	-3.839	No	92.391	-0.56	0.046
23	Dasycarpidan-1-methanol, acetate	-3.831	Yes	91.325	0.235	0.169
24	Methyl oleanolate	-6.156	No	95.022	-0.018	0
25	Swertanone	-6.6	No	97.463	0.692	0
26	Elliptinone	-4.339	No	88.841	0.047	0.06
27	Corialstonine	-3.609	No	97.563	-0.418	0.111
28	β-sitosterol	-6.773	No	94.464	0.781	0
29	α-amyrin acetate	-6.67	No	96.174	0.599	0
30	Lanosterol	-7.498	No	93.119	0.683	0

The herbal components were analyzed in sequence based on their most favorable (maximally negative) binding energy *viz*., chelidonine, akuammigine, echitamine, dihydroberberine, stylopine, arjunone, pareirubrine a, fumariline, corylifol a, corylifolinin, syringaresinol, akuammicine-n-oxide, tocopherol, myrrhanol, isofucosterol, sanguinarine, 9,10-di (methylthio) hexadecanoic acid, methyl ester, di-n-decylsulfone, 16-dehydropregnenolone, stigmasterol, digitoxigenin, bavachin, dasycarpidan-1-methanol, acetate, methyl oleanolate, swertanone, elliptinone, corialstonine, β-sitosterol, α-amyrin acetate, lanosterol, campesterol, cholesterol and α-amyrin.

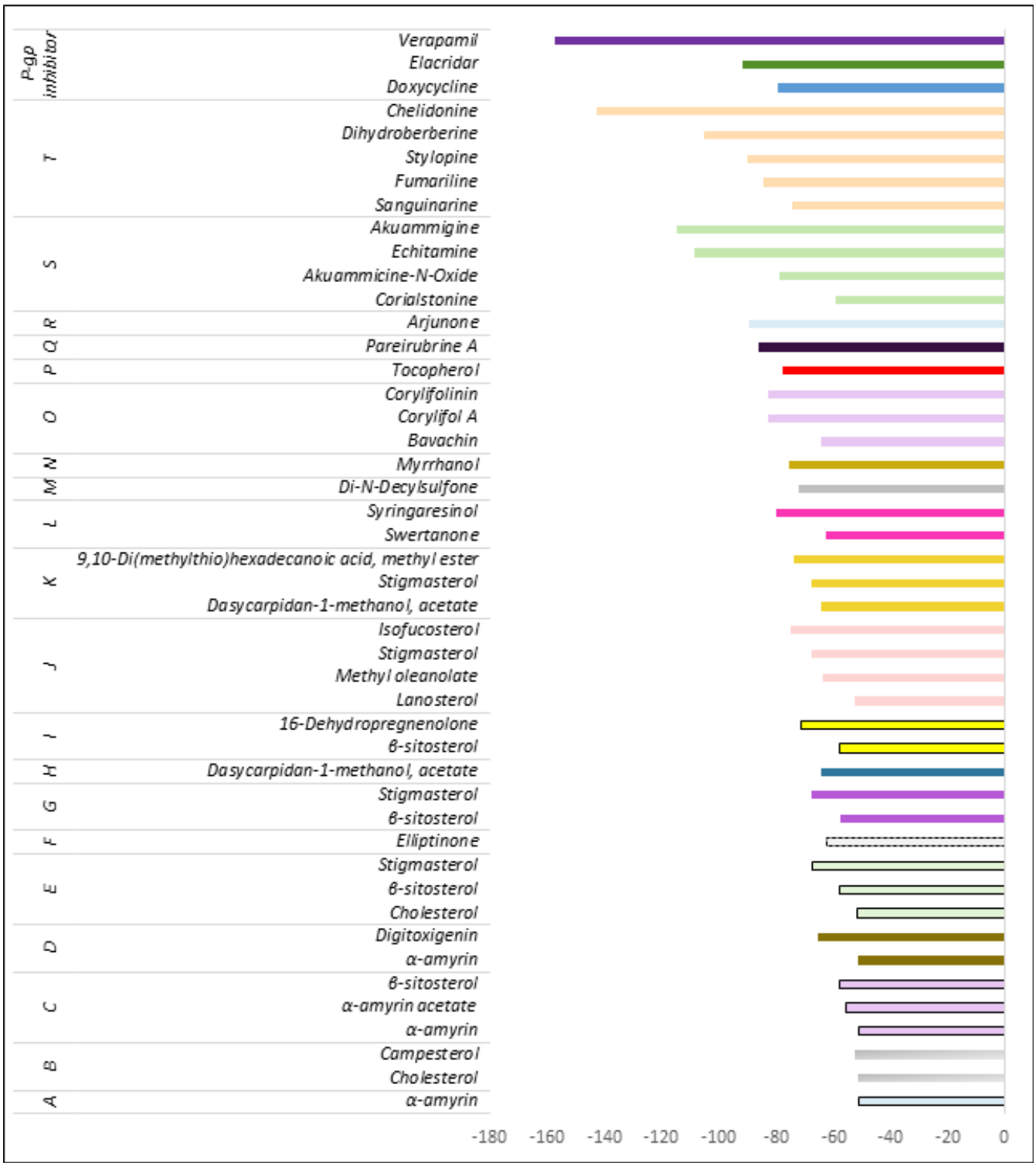


Figure 1. Binding energy of p-glycoprotein inhibitors in immunomodulatory herbs.

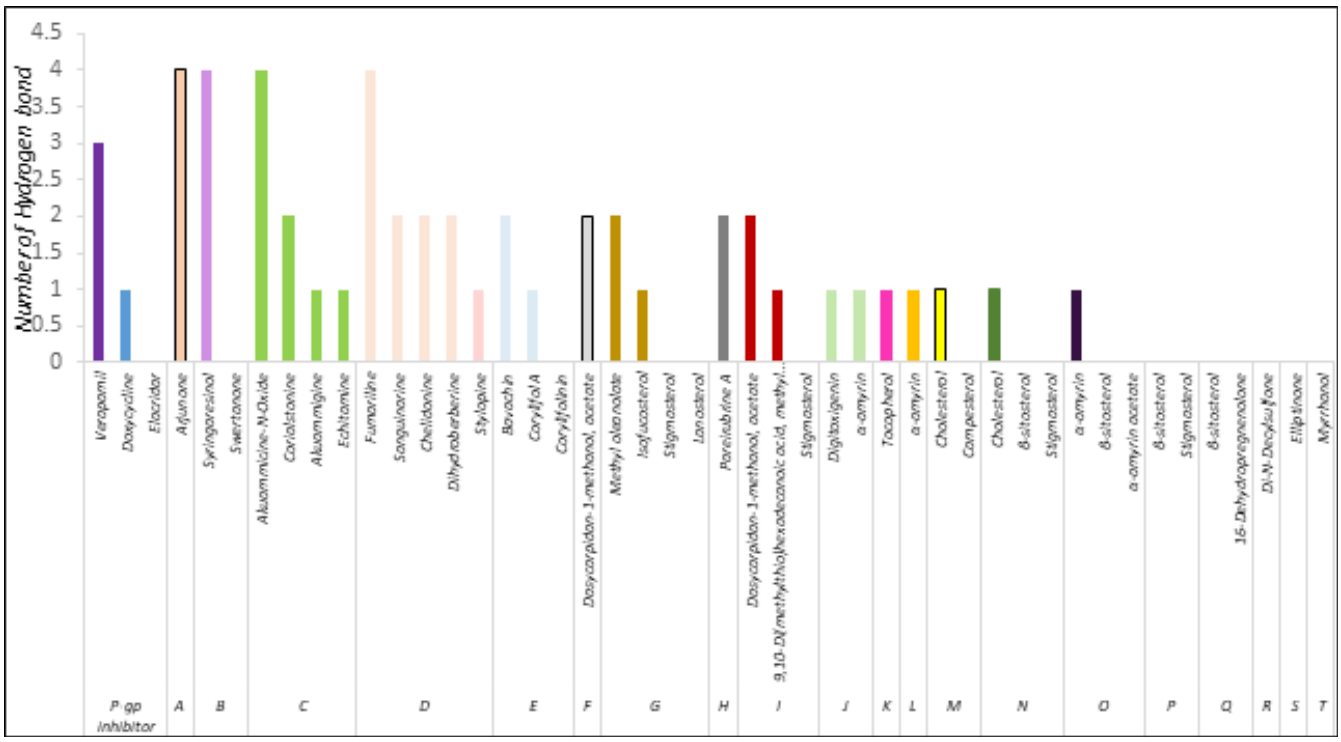


Figure 2. Number of hydrogen bonds of p-glycoprotein inhibitors in immune modulatory herbs.

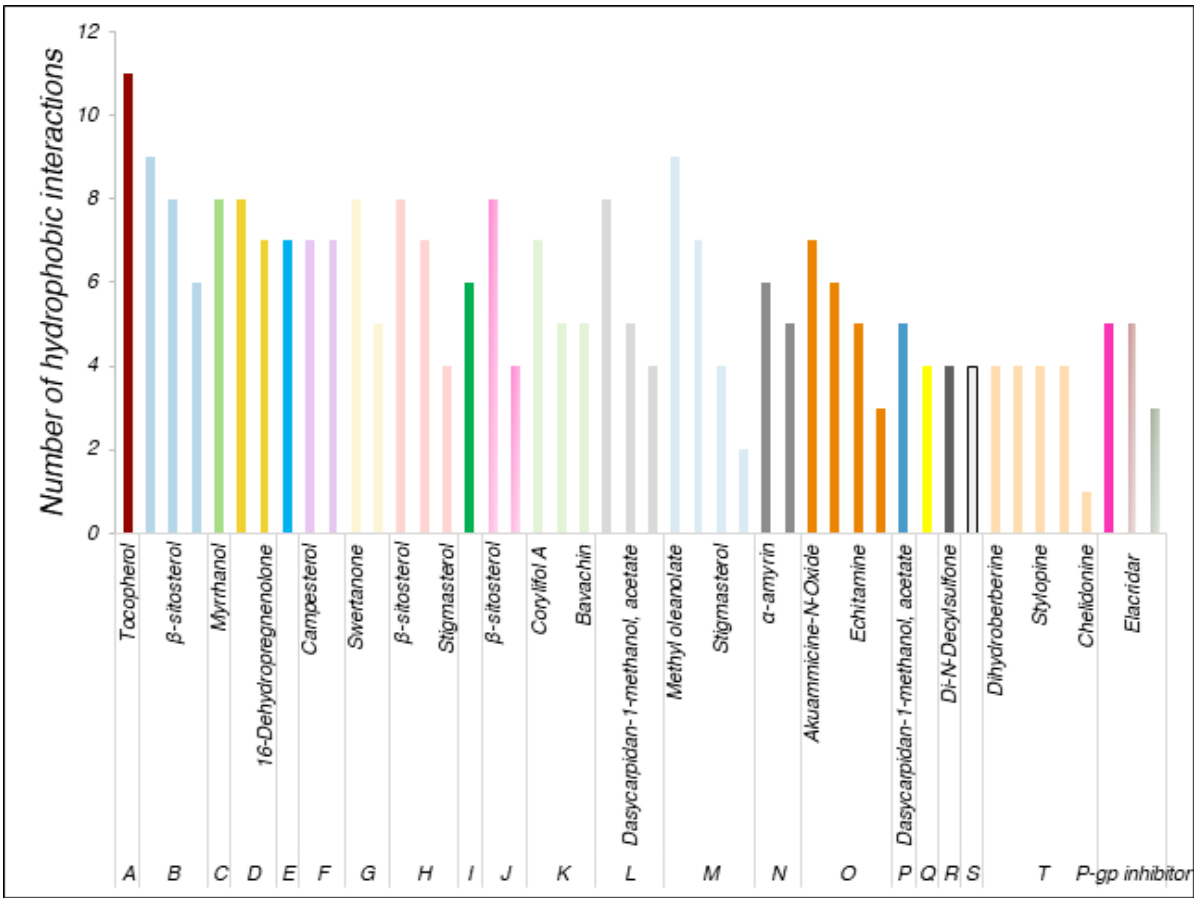


Figure 3. Hydrophobic interactions of p-glycoprotein inhibitors in immune modulatory herbs.

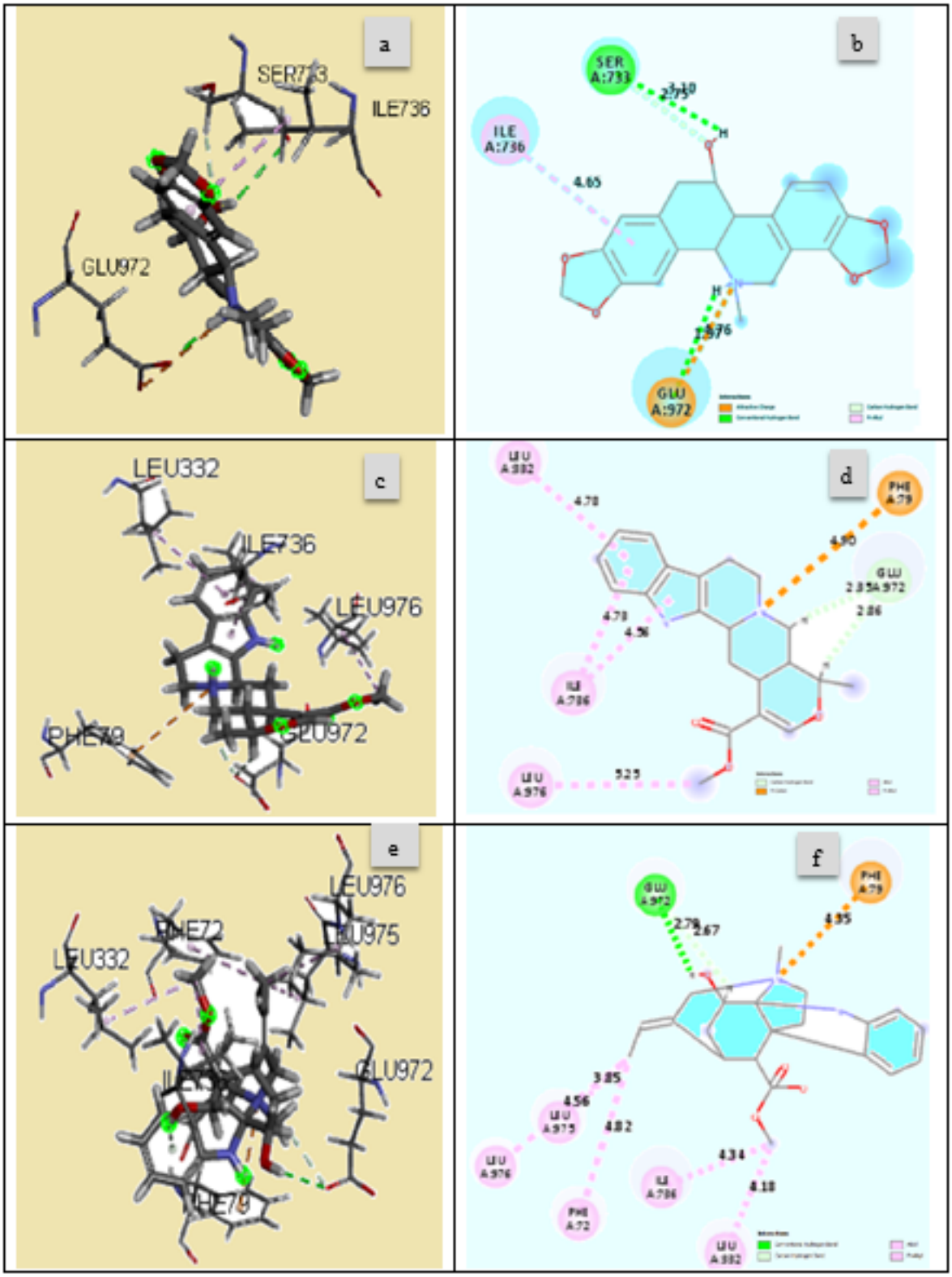


Figure 4. 3D (left panel) and 2D (right panel) docking interactions of natural inhibitors : **(a-b).** Chelidonine; **(c-d).** Akuammigine **; (e-f).** Echitamine with human p-glycoprotein.

The best binding energy of interaction was found to be with chelidonine (-142.52 Kcal/mol), a component present in *C. majus*, followed by akuammigine (-114.79 Kcal/mol) and echitamine (-108.49 Kcal/mol), components present in *A. scholaris*. The average energy of interaction of the inhibitors of *C. majus viz*., chelidonine (-142.52 Kcal/mol), sanguinarine (-74.1 Kcal/mol), stylopine (-89.9 Kcal/mol), fumariline (-84.25 Kcal/mol) and dihydroberberine (-105.09 Kcal/mol) with p-glycoprotein was -99.17 Kcal/mol.

To analyze the specificity and strength of ligand binding with the receptor protein, calculations were performed for hydrogen bonds and hydrophobic interactions. Figure 2 shows the number of hydrogen bonds of p-glycoprotein inhibitors in immune modulatory herbs *viz*., **(A)** *T. arjuna* **(B)** *S. chirata* **(C)** *A. scholaris* **(D)** *C. majus* **(E)** *P. corylifolia* **(F)** *P. marsupium* **(G)** *Ficus religiosa* **(H)** *C. pareira* **(I)** *A. indicum* **(J)** *N. odorum* **(K)** *V. negundo* **(L)** *E. alba* **(M)** *A. barbadensis* **(N)** *B. serrata* **(O)** *B. diffusa* **(P)** *F. racemosa* **(Q)** *H. indicus* **(R)** *C. roseus* **(S)** *P. zeylanica* **(T)** *C. wightii* (in sequence of their efficiency).

The components, when analyzed based on their maximum hydrogen-bonded interactions, were found to be arjunone, syringaresinol, fumariline, akuammicine-n-oxide, bavachin, sanguinarine, chelidonine, dihydroberberine, methyl oleanolate, corialstonine, dasycarpidan-1-methanol, acetate, pareirubrine a, alpha-amyrin, corylifol a, cholesterol, tocopherol, stylopine, isofucosterol, akuammigine, echitamine, digitoxigenin and 9,10-di(methylthio) hexadecanoic acid, methyl ester. Maximal hydrogen bond interaction detected was 04 in arjunone from *T. arjuna*, syringaresinol from *S. chirata,* fumariline from *C. majus* and akuammicine-n-oxide from *A. scholaris.* Chelidonine from *C. majus* exhibited two hydrogen bond interactions.

Figure 3 depicts the *hydrophobic* interactions of p-glycoprotein inhibitors in immune modulatory herbs *viz*., **(A)** *V. negundo* **(B)** *B. diffusa* (C) *C. wightii* **(D)** *H. indicus* **(E)** *P. zeylanica* **(F)** *A. barbadensis* **(G)** *S. chirata* **(H)** *B. serrata* **(I)** *E. alba* **(J)** *F. racemosa* **(K)** *P. corylifolia* **(L)** *A. indicum* **(M)** *F. religiosa* **(N)** *N. odorum* **(O)** *A. scholaris* **(P)** *P. marsupium* **(Q)** *T. arjuna* **(R)** *C. roseus* **(S)** *C. pareira* **(T)** *C. majus* (in sequence of their efficiency).

The components, when analyzed based on their maximum hydrophobic interactions were observed to be tocopherol, alpha-amyrin acetate, methyl oleanolate, beta-sitosterol, myrrhanol, swertanone, 9,10-di (methylthio) hexadecanoic acid, methyl ester, corylifol a, campesterol, cholesterol, isofucosterol, akuammicine-n-oxide, 16-dehydropregnenolone, elliptinone, α-amyrin, corialstonine, bavachin, corylifolinin, syringaresinol, echitamine, dasycarpidan-1-methanol, acetate, digitoxigenin, stigmasterol, sanguinarine, stylopine, fumariline, dihydroberberine, arjunone, di-n-decylsulfone, pareirubrine a, akuammigine, tembetarine, lanosterol and chelidonine. The maximum number of hydrophobic interactions observed was 11 in tocopherol from *V. negundo.* Chelidonine from *C. majus* showed 1 hydrophobic interaction.

Docking utilizing CDOCKER program of Discovery studio (version 2019-21) revealed that chelidonine, akuammigine and echitamine may act as best inhibitors of the efflux protein. In the interaction between p-glycoprotein and chelidonine, two hydrogen bonds were detected with serine733 and glutamic acid 972 at distances of 2.75 Å and 1.97 Å, respectively and one hydrophobic (pi-alkyl) interaction was observed with isoleucine 736 at distance of 4.65 Å. Akuammigine formed one hydrogen bond with glutamic acid 972 (2.86 Å) and three hydrophobic interactions (two pi-alkyl and one alkyl) with leucine 332 (4.78 Å), leucine 976 (5.25 Å) and isoleucine 736 (4.73 Å). Similarly, echitamine participated in a hydrogen bond interaction with glutamic acid 972 (2.79 Å) and exhibited five hydrophobic interactions (pi-alkyl and alkyl) with phenylalanine 72 (4.82 Å), leucine332 (4.18 Å), leucine 975 (3.85 Å), leucine 976 (4.56 Å) and isoleucine 736 (4.34 Å) (Figure 4). Chelidonine was identified as the best compound among 379 components, primarily based on maximum binding affinity with p-glycoprotein. It was thus simulated to obtain more detailed and accurate energies and trajectories to comprehend the binding mechanism.

3.4 Molecular Dynamics Simulation

To confirm the efficacy of chelidonine as a potential inhibitor and to evaluate the stability of p-glycoprotein-chelidonine complex under dynamic conditions, MD simulation was conducted using GROMACS for 40

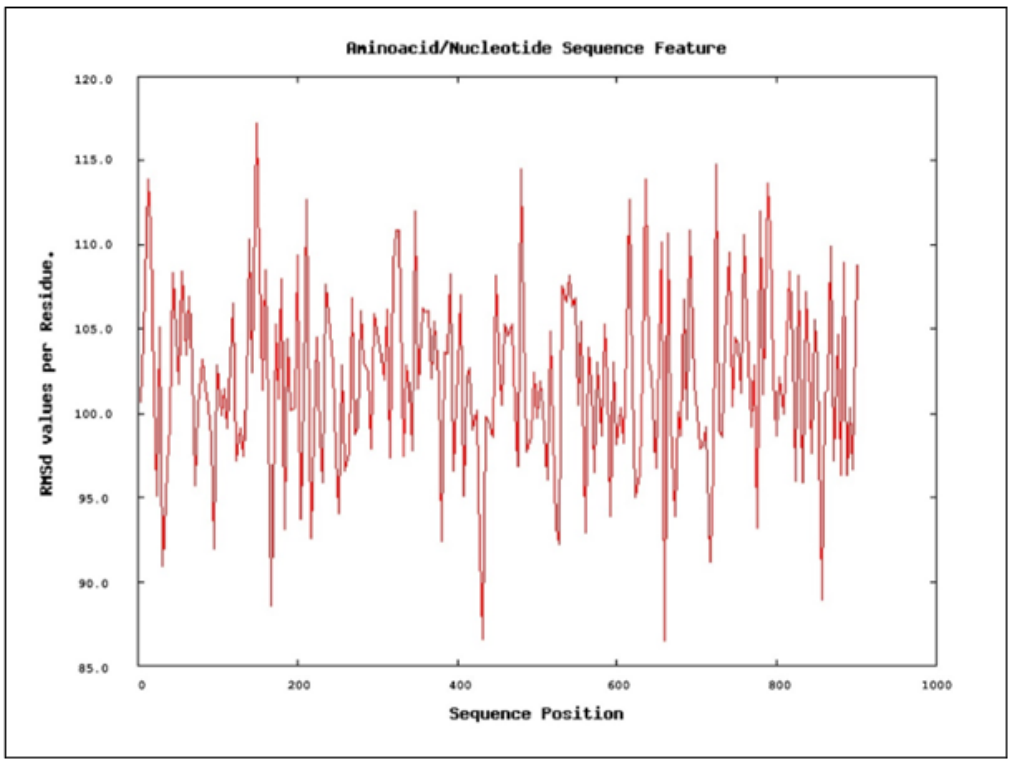


Figure 5. Root mean square deviation curve of p-glycoprotein-chelidonine complex.

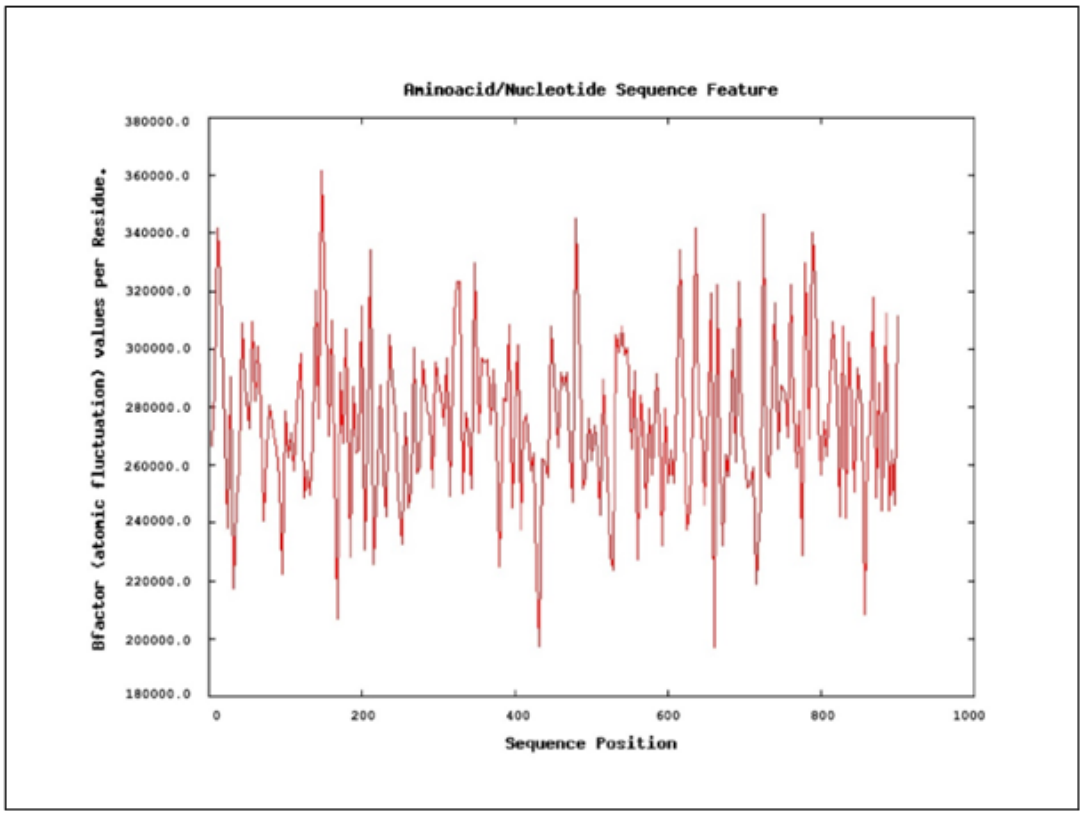


Figure 6. B factor graph of p-glycoprotein-chelidonine complex.

nanoseconds. The best conformation was acquired from the result of molecular docking analysis using CDOCKER and subjected to MD simulation. The stability of the docked complex of p-glycoprotein and chelidonine was investigated by RMSD value per residue (Figure 5). There was uniformity in RMSD fluctuation in context of amino acid residue of the protein when the protein is bound with the ligand.

B factor is another important criterion for understanding the stability of the complex. It represents the mean squared displacement of each atom from its average position over time. The uniformity in B factor graph is an indication that overall stability of p-glycoprotein-chelidonine complex is being maintained throughout the duration of simulation (Figure 6). Therefore, the interaction of chelidonine with p-glycoprotein is stable and corresponding function of the small molecule should be imparted.

For p-glycoprotein-chelidonine complex evaluated, the TIPS3BOX solvent model was implemented successfully with a box size of 10 (Figure 5 and 6) and this resulted in stable B factor and RMSD which indicated that the simulation was successful and the complex was stable, depicting binding efficiency of the ligand which we have earlier reported in the interactions data.

4. Discussion

Efflux transport is a common cause of therapeutic failure in multiple chronic diseases, including kidney diseases[38]. Over expression of p-glycoprotein causes a decrease in the intracellular accumulation of drugs, ultimately leading to the development of MDR with reduced drug efficacy. Concurrent administration of p-glycoprotein inhibitors along with substrate drugs can prevent the drug expulsion and render therapeutic benefit in treated patients[39].

Phytochemicals synergistically interact with drugs and overcome resistance via modulation of p-glycoprotein transport function[40]. They demonstrate significant immune modulatory effects by interacting with p-glycoprotein and various cellular pathways, which are vital in immune response regulation. Stigmasterol from *F. religiosa* inhibits p-glycoprotein-mediated rhodamine 123 efflux[41], potentially enhancing immune cell response, while syringaresinol from *S. chirata* enhances IL-12 secretion in mouse lymphoid and myeloid type dendritic cells[42], supporting immune activation. Similarly, guggulsterone from *C. wightii* exhibits anti-inflammatory effect by down regulating iNOS and NF-κB activity in lipopolysaccharide-stimulated cells[43]. Chelidonine from *C. majus* also modulates inflammatory pathways, *viz.*, IL-1β-mediated NF-κB activation, to prevent cartilage degeneration and synovial inflammation in osteoarthritis[44]. This suggests that herbal components have the potential to support immune modulation through p-glycoprotein interactions and cytokine pathway modulation, aiding in the regulation of inflammation.

The herbal inhibitors may be arranged based on their lowest binding free energy, as well as hydrogen bonding and hydrophobic interactions. These factors are crucial in stabilizing the appropriate pose of the ligand at the binding site of the receptor protein. Hydrogen bonding confers stability to the protein structure and imparts selectivity to protein-ligand interactions[45]. The hydrophobic interactions are entropy-driven and also play crucial role in ligand binding[46]. Hydrophobic interactions are assessed based on the extent of buried hydrophobic surface area during ligand binding. This structural parameter demonstrates the strongest correlation with binding free energy[47]. Binding affinity and interaction were found to be strongest in case of chelidonine from *C. majus*. This is apparent from its lowest binding energy of interaction (-142.52 Kcal/mol).

The accuracy of the input structure is of paramount importance in MD simulation because minor inaccuracies can trigger instability or yield unrealistic trajectories. The primary objective of initial structure validation is to identify and address common issues in the input for MD simulations. Additionally, structure validation facilitates the selection of specific system fragments for simulation, particularly in cases where alternative models or multiple subunits exist within the incoming structure. The evaluation involves a compilation of potential options for selecting system parameters to be simulated. These options encompass the structure model, structure chains, alternate residue or atom locations, non-consecutive residues or sequence gaps, various atom clashes (including steric clashes, alpha-carbon clashes, clashes involving polar donors and acceptors, clashes with a polar atoms and ionic clashes with positive or negative charges), improper

chirality, unusual peptide-bond cis configurations and disulfide bonds. The complex used for the analyses passed the major checkpoints of the evaluation scheme.

This is hitherto the first work on molecular docking utilizing CDOCKER program of discovery studio to explore the interactions between natural inhibitors *viz*., chelidonine, akuammigine and echitamine, with the 6C0V structure of p-glycoprotein. This is also the first MD simulation study on p-glycoprotein-chelidonine complex carried out for a duration of 40 nanoseconds with the utilization of GROMACS software. The uniformity in RMSD fluctuation along with uniformity in B factor graph observed during MD simulation reflect stability of chelidonine-p-glycoprotein complex and confirm chelidonine inhibitory effect. Moreover, chelidonine has been reported to enhance the immune system circumventing immunosuppression during chronic illnesses[48].

Verapamil is an established inhibitor of p-glycoprotein[49]. The binding energy of verapamil (-157.24 Kcal/mol) is comparable with that of chelidonine (-142.52 Kcal/mol) at the same binding pocket. It exhibited three hydrogen bond interactions with leucine 332 (2.36 Å), leucine 975 (2.94 Å), isoleucine 736 (2.59 Å) and five hydrophobic interactions with phenylalanine 732 (4.46 Å), phenylalanine 336 (5.63 Å), phenylalanine 79 (4.59 Å), alanine 729 (4.90 Å), isoleucine 736 (5.17Å) respectively which is comparable with two hydrogen bond interactions in case of chelidonine. However, the common adverse effects that occur with verapamil consumption include constipation, nausea, headache and edema. In addition, verapamil-induced hepatic injury has also been reported in some cases[50,51]. On the other hand, chelidonine was found to have better binding affinity (-142.52 Kcal/mol) than other established inhibitors of p-glycoprotein such as doxycycline[52] and elacridar[53] whose binding energy was determined to be -79.09 Kcal/mol and -91.59 Kcal/mol respectively. Doxycycline established a single hydrogen bond with leucine 976 (3.74 Å) and engaged in three hydrophobic interactions with leucine 332 (3.96 Å), isoleucine 736 (4.52 Å) and phenylalanine 732 (4.63 Å). Elacridar demonstrated five hydrophobic interactions with phenylalanine 732 (5.38 Å), phenylalanine 72 (6.80 Å), leucine 332 (6.42 Å), isoleucine 328 (5.76 Å) and alanine 80 (5.84 Å). Thus, chelidonine, an immune enhancer, with its better binding affinity than doxycycline and elacridar, as well as no significant cytotoxicity[54] and hepatotoxicity[55], may be regarded as the lead compound in modulating the efflux of immunomodulatory drugs mediated by p-glycoprotein.

Verapamil, a well-known calcium channel blocker and p-glycoprotein inhibitor, alters immune cell activation and cytokine secretion, impacting T-cell and macrophage function[49]. Doxycycline, an antibiotic with anti-inflammatory properties, inhibits matrix metalloproteinases and reduces pro-inflammatory cytokines, modulating immune responses in inflammatory diseases[52]. Elacridar, a potent p-glycoprotein inhibitor, affects immune cell activation and cytokine release[53]. All three drugs modulate p-glycoprotein, influencing immune responses and overcoming resistance to immune therapies, while also suppressing inflammation and regulating cytokine production.

ADMET predictions are specifically developed to assess the pharmacokinetic properties. Such study of the immune modulatory herbs revealed that some of the components inhibited the function of the transporter which would enhance the bioavailability of co-administered drug inside the human system. This indicates that the drug substrates of p-glycoprotein which are being effluxed by the transporter would be retained in the cells, thereby enhancing therapeutic efficacy.

5. Conclusion

The present study elucidated the possible inhibitory activity of these natural compounds on p-glycoprotein which would allow retention of the immuno modulatory drugs that are substrates of the efflux transporter. These herbal compounds with significant binding interactions with 6C0V structure exhibited promising drug-likeness and ADMET attributes suggesting their candidature as potential lead molecules for co-administration along with allopathic drugs. Chelidonine is thus the lead compound, followed by akuammigine and echitamine as analyzed from a pool of immuno modulatory herbs which might play a crucial role in amelioration of MDR *via* p-glycoprotein, thus increasing the bioavailability of the substrate allopathic drugs. Further *in vitro* and

in vivo investigations may be required to verify the pharmacological importance of these lead molecules.

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7. Author Contributions

Conceptualization, P.G.; methodology, P.G. and S.G.; software, S.G.; validation, C.R. and P.G.; formal analysis, C.R.; investigation, C.R.; resources, P.G.; data curation, C.R. and S.G.; writing—original draft preparation, C.R.; writing—review and editing, C.R. and S.G.; visualization, C.R. and P.G.; supervision, P.G. All authors have read and agreed to the published version of the manuscript.

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