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Original Research Article

An integrated computational study for screening medicinal plant phytochemicals for possible inhibitors of the human bile salt exporter pump

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ABSTRACT

Background: The primary carrier of bile acids in humans is the bile salt export pump (BSEP). which are crucial for the digestion and absorption of fat. Type 2 progressive familial intrahepatic cholestasis (PFIC-2), which is brought on when BSEP is suppressed and causes a decrease in bile flow and a buildup of cytotoxic bile salts in the liver, is one of the main causes of cholestasis in Saudi Arabia. Elucidate the inhibitory potential with minimal or no adverse effects.
Methods: The structure (6LR0) was downloaded from the PDB. Protein active sites were anticipated because these are pockets where ligands can bind and perform reactions to treat an infection. The PubChem zinc and mpd3 databases were used to get the ligands' structures. Molecular operating environment (MOE) was utilized to perform molecular docking of 1600 phytochemicals against BSEP. LigX was used to observe the docking hits for interaction analysis.
Results: identification of 4 potential candidates for binding to the BSEP active site. then, Protox II-was used to forecast toxicity for the selected hits. Molecular dynamics simulations were also used to assess the binding complex's stability in water for 100 nanoseconds. The strong binding affinity of high-ranked drugs was predicted by our molecular docking and simulation.
Conclusions: This approach could be useful in determining the efficiency of a therapeutic molecule in the therapy of the BSEP. The aim of this research is to identify novel -BSEP drug targets, and in future in vitro and in vivo research could prove its clinical efficiency.

Keywords: BSEP, Molecular docking, Phytochemicals, MOE, ADMETsar

INTRODUCTION

The bile salt export pump (BSEP, ABCB11) is a canalicular-specific exporter expressed primarily in hepatocytes' cholesterol-rich apical membrane.[1] The release of bile salts from the liver into the bile canaliculi is easier by BSEP.[2,3] Bile acids are mainly responsible for promoting dietary fat digestion and absorption by forming micelles.[4] Aside from that, they are increasingly being demonstrated to have hormonal effects all over the body.[5,6] Variations in the ABCB11 gene are associated with various distinct types of progressive familial intrahepatic cholestasis (PFIC).[7,8] PFIC is defined as the start of cholestasis at an early age, which progresses to liver cirrhosis and failure.[9-11]

The ABCB subfamily has a wide range of ABC proteins, including dimeric half transporters with monomers containing one TMD and one NBD, as well as full-length transporters with all four domains fused into a single polypeptide chain. ABCB6, ABCB7, ABCB8, ABCB9, and ABCB10 are homodimers, while ABCB2/ABCB3 is a heterodimer. ABCB1, ABCB4, ABCB5, and ABCB11 are the four full-length transporters in the ABCB subfamily. The heterodimeric ABCB2/ABCB3 and full-

length ABCB11 members differ in that they only have one canonical NBS instead of two.[12]

NSAIDs, anticonvulsants, antibiotics, antipyretics, and other medications, depending on the patient's state, are the sole options for symptomatic therapy of BSEP infection as of yet. Although vaccines are available for prevention, not all clinical isolates respond well to them, and some people can experience negative medication reactions. In order to cure BSEP defects, new chemical entities must be discovered, or existing molecules must be explored.

Plants are still used as a traditional form of healthcare against some disorders in the modern world of medicine.[13] By creating specific compounds or secondary metabolites that are non-nutritive but effective in defense mechanisms, plants can defend themselves against pathogenic microbes, dangerous insects, and severe environmental changes.[14] The metabolites can act as a direct or indirect defense mechanism against infections or hazardous conditions since they possess anticarcinogenic, anthelmintic, anticarcinogenic, antigenotoxic, antiproliferative, anti-inflammatory antimutagenic, and antioxidative.[15] Various studies examine the BSEP's inhibitory effect while employing an in-silico technique which supports our study.[16]

Development of novel medications is required to persuade a large number of therapeutic applications in a large number of disease implications. Scientists are currently developing a big amount of 3D structural data as well as a large number of medicinal lead compounds. As a result, drug development includes virtual screening of pharmaceuticals, molecular docking, and MD simulation studies to deal with such a large set of data and design new therapeutic drugs. The protein BSEP was the target of the current study's therapeutic candidates. To determine whether phytochemicals were suitable as inhibitors, they were docked with the catalytic triad of the BSEP protein. Based on a minimal S score, RMSD value, and good interactions, the four best complexes were chosen out of 1600 best compounds. The virtual compounds library is also pre-screened using an in silico molecular simulation method. We found natural chemicals based on BSEP inhibition in this investigation and the top inhibitor lead compounds. *In vitro* and *in vivo* biological assays can be used to examine further the potential hit the chemicals discovered in this investigation.

METHODS

Preparation of target protein

This study was conducted form January-2022 to April-2022, at Najran university hospital, the protein with PDB ID (6LR0) was obtained from the protein data bank with 3.50 resolutions to perform molecular docking.[17] The protein was prepared for molecular docking by removing water molecules, metal ions, and cofactors, adding charge, hydrogen atoms, and utilizing MOE to reduce the energy of 3D structures.[18]

Library preparation

Employing in silico approaches, a thousand known phytochemicals were picked from multiple databases, including PubChem, MPD3, and Zinc, and their potential inhibitory impact on the target proteins was assessed.[19-21] The plant-based phytochemicals were selected depending upon their pharmacological effects, according to the literature review. Flavonoids, alkaloids, and sterols were the most common phytochemicals used. The MOE program was used to generate a ready-to-dock library of the chosen phytochemicals. The selected ligands' two-dimensional (2D) chemical structure was drawn using ChemDraw.[22] Before using the MOE ligand database, the compounds were refined with protonate3D and the energy was minimized.

Binding site

A protein-ligand binding site is a pocket-like area of a protein that binds a ligand. This protein-ligand binding region is called an active site because it is responsible for protein function. A ligand interacting with a receptor-ligand binding site often changes the protein's shape, affecting cellular function. To function properly, a protein's binding site must be a significant component of signal transduction pathways.[23] The binding site will be used to determine whether or not an interaction occurs at a significant portion of the protein's surface. By utilizing MOE, it was possible to identify the active site on the target protein molecule.

Drug scan /ADMET properties

The toxicological and pharmacological features of all ligand structures were investigated using the Admetlab 2.0 and SWISSADME servers to forecast absorption, distribution, metabolism, excretion, and toxicity (ADMET).[24,25] Carcinogenicity, the AMES test, Physical and chemical properties were evaluated, including molecular weight (MW), hydrogen bond acceptors(HBA), the log p (miLogP), partition coefficient of octanol and water (miLogP), and hydrogen bond donors.[26] The draggability of the top-docked ligands was evaluated with the Molinspiration online tool. Drug scan of phytochemicals was executed using Lipinski's rule of five phytochemicals that follow these five rules are favorable for further study as these phytochemicals are not harmful for body.[27,28]

Molecular docking

MOE software was used to perform molecular docking in this study. The MOE is a docking tool that predicts the interaction between small compounds such as substrates or drug candidates and receptors with known 3D

structures. To build a minimum energy structure, the MOE software used the "Triangular Matcher" technique as the default ligand placement strategy. The London dG scoring algorithm in MOE was utilized to rescore the simulated poses. RMSD values and binding affinity were used to identify top hit compounds and best conformation after docking. The MOE LigX tool was used to display the best-docked complexes and interpret 2D plots of ligand-receptor interactions.[29] The interfaces Discovery studio was used to visualize docked buildings in 3D.[30]

MD simulation

AMBER18 was utilized to perform MD simulations of the docked solutions.[31] Each top complex was explicitly solvated with water molecules, and then counter ions were added to create a neutral system. Following that, a water box with walls 12 Å away from the protein surface was created using the TIP3P solvent model to encircle the complex.[32] The complex was simulated using periodic boundary conditions, with electrostatic interactions modeled using the particle–mesh Ewald procedure. For nonbounded interactions, a threshold value of 8 Å was defined during the procedure. Water molecules were minimized for 500 cycles, then the entire system was minimized for 1000 rounds. The temperature of each system was then steadily increased to 300 K. Solutes in the first phase were restrained for 50 ps during equilibration of counterions and water molecules, while protein side chains were relaxed afterward. At 300 K and 1 atm, a 50 ns MD simulation was performed for two Fs under the NPT ensemble. While the SHAKE algorithm constrained covalent and hydrogen bonds, Langevin dynamics used to control system temperature. Original structure used as reference and AMBER's CPPTRAJ used to generate an RMSD (root mean square deviation) plot to assess the system's MD simulation convergence.[33,34] Structural flexibility of ligands was calculated using ligand RMSD. In a given set of dynamics, RMSF reflects the root mean square averaged distance between an atom and its average geometric position.[35]

RESULTS

Database screening and molecular docking

The purpose of molecular docking methods is to predict the best binding mode of a ligand-receptor protein. The top four docked poses were chosen from 1600 docked molecules. A good docking approach must be able to predict the native ligand pose inside the receptor-binding site (i.e., locate the experimental ligand geometry within a certain tolerance range) as well as the physical-chemical molecular interactions that go with it. Furthermore, while studying huge compound libraries, the approach must be able to identify binding from non-binding molecules and appropriately rank these ligands among the database's top compounds. The ranking parameters included standards-based on thresholds that needed a ligand to bind to all of the chosen N protein targets, engaging all of the binding pocket's hotspot conserved residues, and showing good binding affinity values. Chloroquine, hydroxychloroquine, Arbidol, and Remdesivir were top 4 phytochemicals based on binding affinity (Table 1).

Chloroquine and hydroxychloroquine have binding affinities of 15.27 and -12.27 kcal/mol, respectively, and establish hydrogen bonds with the side chains/backbones of Arg696, Lys700, Glu 381, and Ser377, respectively, while additional close interacting residues were Ser264, Val263 Ala257, Lys700, and Ser263 shown in (Figure 1).

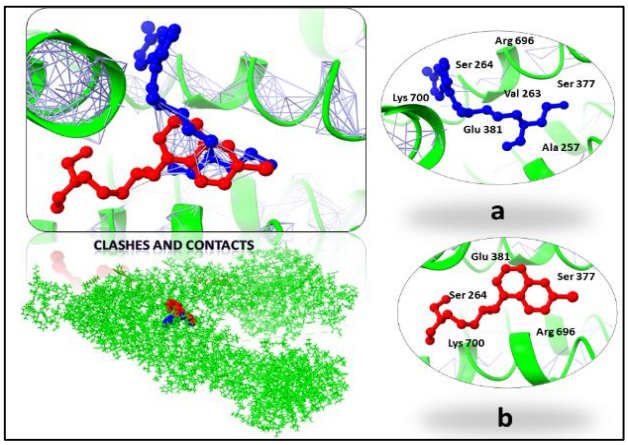

Figure 1 (A and B): Docked complexes showed details of interacting residues of chloroquine and hydroxychloroquine.

With a binding affinity of 10.95 and 9.87 kcal/mol, respectively, arbidol and remdesivir (Figure 2) demonstrated substantial interaction with active site residues. Interactions between Arg 696, Ser 377, and pi stacking. Key residues involved in forming the interactions between the top hit ligand conformations and binding pocket of target proteins were Gly260, Leu703, Leu706, Val707, Ala370, Gly374, Val329 with conserved Arg696, Ly700, Glu381 and Ser377 interactions, all 4 ligands showed significant hydrogen binding.

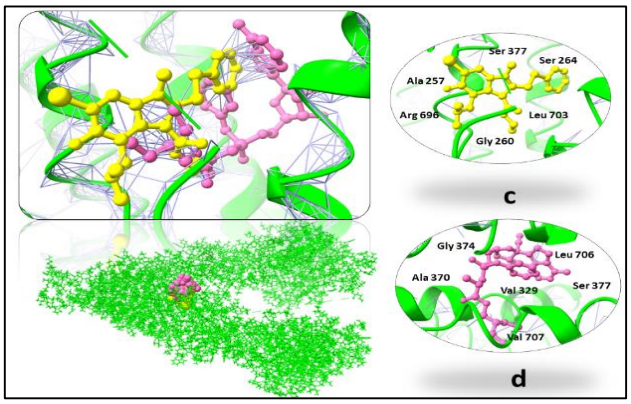

Figure 2 (A and B): Docked complexes showed details of interacting residues of arbidol and remdesivir.

Drug scan/ ADMET profiling

The selected phytochemicals utilized in this research met the requirements for being prospective therapeutic candidates, together with molecular weight, HBA, HBD, and logp as shown in (Table 2). The ADMETsar was utilized to predict the ADMET-associated properties of the lead compounds. All phytochemicals tested were nontoxic and noncarcinogenic, indicating that they could be used as medication candidates. Due to poor pharmacokinetics and toxicity, many medicines do not exploit this mechanism in their development. Early drug development relies on high-performance ADMET profiling studies to identify active lead compounds. The ADMET characteristics of the derived compounds for all targets were shown in supplementary file.

Table 1: Lead bioactive phytochemicals along with binding affinity and interacting residues.

Compounds ID	Compounds names	Binding affinity (kcal/mol)	RMSD	Interacting residues
2719	Chloroquine	-15.27	0.76	Arg 696, Lys 700, Glu 381, Ser 377, Ser 264, Val 263, Ala 257
3652	Hydroxychloroquine	-12.27	0.56	Arg 696, Lys 700, Glu 381, Ser 377, Lys 700, Ser 264
131411	Arbidol	-10.95	1.23	Arg 696, Ser 264, Ser 377, Gly 260, Leu 703, Ala 257
121304016	Remdesivir	-9.87	1.96	Ser 377, Leu 706, Val 707, Ala 370, Gly 374, Val 329

Table 2: Drug like parameters of lead compounds.

Compounds	PubChem ID	MW(g/mol)	H-donor	H-acceptor	Log p	TPSA (Å)
Chloroquine	2719	320.89	2	1	3.39	29.36
Hydroxychloroquine	3652	336.89	3	2	2.37	49.59
Arbidol	131411	477.42	1	3	6.07	54.70
Remdesivir	121304016	602.58	4	10	1.24	203.01

Molecular dynamic simulation

The MD simulation technique is an effective method in the field of biophysical research, and it provides significant dynamic values of protein-ligand interactions. Several studies demonstrated that MD simulations are necessary for particular systems in order to determine the precise binding conformations; as a result, this technique has a significant amount of weight in the field of computer-assisted drug development. On the top model that was created by docking with chloroquine inhibitor, MD simulations were carried out so that the present investigation could be carried out. For the purpose of elucidating the dynamic stability and ensuring the rationality of the ligand sampling, the relative mean squared deviation (RMSD) values of the BSEP protein and the heavy atoms of the chloroquine inhibitor relative to their own initial structures were calculated, and RMSD trajectories were examined over the course of a time span spanning the entirety of 100 nanoseconds. The RMSF analysis was then used to further compute the stability as well as the residual flexibility of the proteins that were present. These data point to a significant degree of concordance regarding the intermolecular stability. In addition, a Rg analysis was carried out in order to evaluate the structural equilibrium and protein compactness over the course of the simulation. The ideal Rg value for globular proteins should be somewhat low; however, the Rg value for protein forms with a greater number of turns and loops could be substantially higher.

Root mean square deviation

Molecular dynamics simulations of top complexes carried out for 100 ns to clarify the complex binding stability and retrieve receptor structural significant information in binding and that can be modified to enhance binding conformation and, eventually, complex affinity for the target compound. RMSD results revealed that MD simulation was equilibrated between 2.5Å and 3Å at the first jump up to 35 ns. After that, it showed stability at 5.6 Å throughout time period of 100 ns (Figure 3).

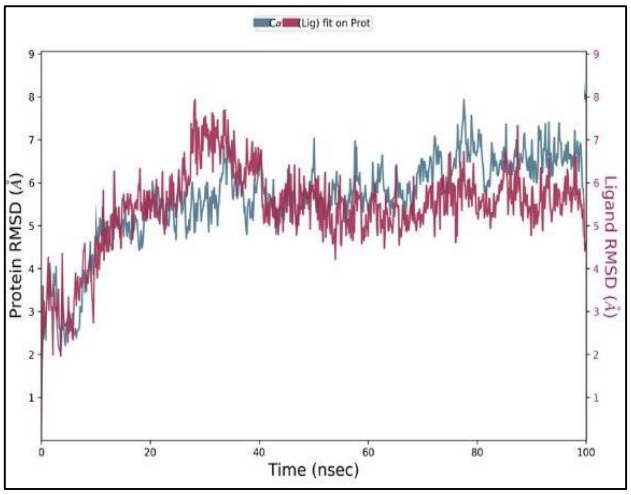

Figure 3: RMSD residual flexibility was shown over 100 ns time.

Root mean square fluctuation (RMSF)

To further evaluate residential versatility over 100 ns, the RMSF have been calculated. To compute the RMSF for single atoms, the trajectory conformation variability can be controlled. The average RMSF of the entire position of compound Chloroquine was 1.2±3.1Å. There were substantial variations in all trajectories at the C-terminal and the N-terminal up to residue number 400. The complete residual variations of complexes are demonstrated in (Figure 4).

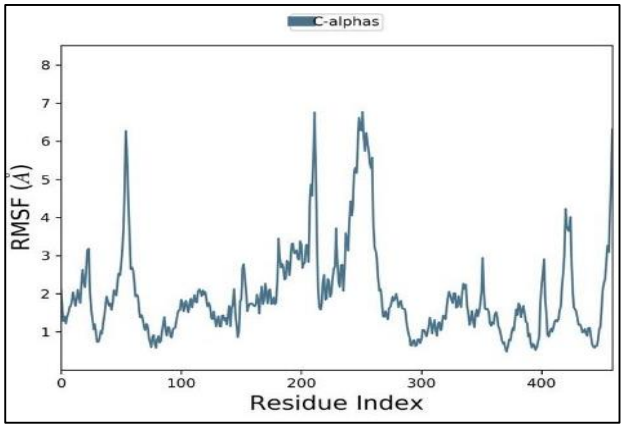

Figure 4: RMSD residual flexibility was shown over 100 ns time.

Solvent accessible surface area and secondary structure element distribution analysis

Solvent accessible surface area (SASA) is a different technique to keep proteins stable and folded. The calculated SASA values for the complex were displayed in (Figure 5 A). The average SASA values for chloroquine was found to be 150 Å^2, 220 Å^2 and 280Å^2, respectively indication that there no notable variations in available area of all systems during simulation process.

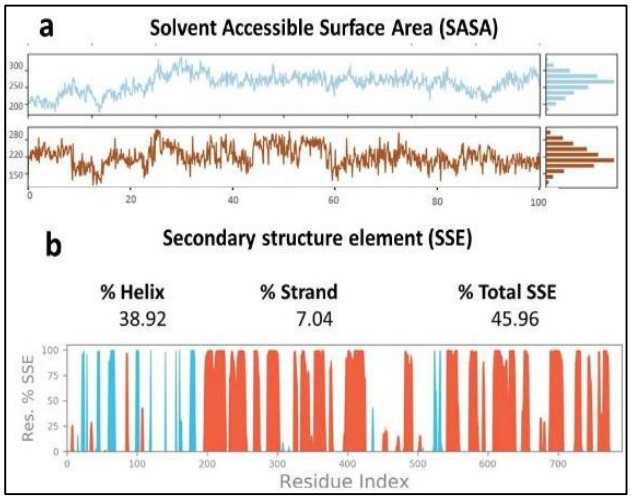

Figure 5: SASA and SSE graphs showed the stability of the protein and it's folding.

To evaluate the protein conformational changes in terms of percentage, total SSE elements, the percentage of helix, and percentage of strands, protein 2D structure elements (SSE) such as α-helices and β-strands were also evaluated all over the simulation process. Graphs for the secondary structure element (SSE) distribution were shown in (Figure 5 B). Graphs showed the SSE distribution by residue index within whole protein structure. Total SSE distribution for complex chloroquine was 45.96% Alfa-helices. From the SSE distribution analysis, it is depicted that all ligands showed stability to protein.

DISCUSSION

The bile salts generated by the liver are transported by BSEP, which is found on the lateral membrane of hepatocytes' bile ducts.[36] BSEP secretes bound bile acid into the bile under normal conditions, and this is the rate-limiting phase in the entire hepatic and intestinal circulation.[37] Defects in the transport function of BSEP cause cholate-dependent bile secretion to be disrupted, resulting in a range of cholestasis disorders and, eventually, liver failure.[38]

The usefulness of medicinal herbs in the treatment and control of contagious diseases has been thoroughly proved. Plant diversity is still valuable for humans since it gives a variety of traditional and modern therapeutics to the healthcare system.[39] Due to naturally occurring chemicals, medicinal plants are regarded as a natural reservoir and an unlimited source of therapeutic medications. Due to their multitarget pharmacological effects, the dazzling array of phytochemicals produced by herbal medicines has attracted the attention of researchers.[40]

In-silico analysis has modified the drug design process by effectively lowering expenditure compared to the traditional drug formation procedure. With developments in the bioinformatics domain, tools and software are being produced for applying these approaches. *In-silico* chemical libraries are now available due to advances in cheminformatics. The characteristics of these compounds were tested for drug similarity using advanced computational approaches.[41]

The compounds having the best residue interaction with the target protein were identified by molecular docking. Out of 1600 docked molecules, the top 4 molecules were selected as the best molecules according to their high binding affinity and drug evaluation. These four molecules: Pubchem2729, Pubchem3652, Pubchem131411, and Pubchem121304016 were selected based on low score, i.e., RMSD<3 and many interacting residues. These phytochemicals showed high binding energy in the range of -15.27 to -9.87 (kcal /mol). These phytochemicals predominantly engaged with the active site residues of Arg 696, Lys 700, Glu 381, Ser 377 and

with other close contact residues Ser264, Val263 Ala257, Lys700 and Ser266.

Molecular properties and drug-likeliness of the selected complexes were estimated according to the "Lipinski rule of Five". This rule states that, the molecular weight of the compound must less than 500 daltons, less than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and AlogP value fewer than 5. All compounds fulfill the Lipinski's rule of five and show no violation. Selected compounds have low scoring values as compared to the standard drugs and have RMSD values less than 3. Each of them upheld the “Lipinski rule of five” and showed no desecration. Assessing the properties of lead compounds is a major challenge in the drug development pathway.[42] Drug development relies on high-performance and quick ADMET profiling assays to identify active drug targets.[43] The absorption of all possible chemicals has no negative effects, according to ADMET analysis. The ADMET qualities of promising compounds for several models such as P-glycoprotein substrates, BBB penetration, and gastrointestinal absorption all yielded favorable results, indicating that the compounds' ability to operate as a therapeutic candidate is strong. Cytochrome P450 (CYP) is a cluster of isozymes comprising fatty acids, bile acids, carcinogens, steroids, and the metabolism of drugs. Fifty-seven CYPs are encoded by human genome, of which fifteen are participating in the xenobiotic chemicals and another drug metabolism.[44] CYP enzymes association is very important for drug metabolism almost 75% of the phase 1 of drug metabolism depends upon its association.[45] Identification of active lead compounds depends upon the high-performance and fast ADMET profiling assays at early drug discovery.[43] ADMET profiling shows that there is no side effect of absorption of all potential compounds

More specifically, our work offers a comprehensive and integrated reorganization strategy of novel flavonoids based on BSEP inhibitors and can serve as a basis for the efficacy of these compounds *in vitro* research. The authorized vibrating conformations over 100 ns MD simulations of identified compounds, which established the technique of producing safe and more efficient BSEP inhibitors, remain combined with the thorough explanation of the interrelating.

The major limitation of molecular docking is due to the lack of confidence on the ability of scoring functions to give accurate binding energies.

CONCLUSION

The goal of this research is to find compounds in natural products that can reduce BSEP activity. The docked library of (1600 compounds) was screened, and the top four compounds with strong binding affinity were identified as potential inhibitors of BSEP activity, according to the results. MD simulation of top chloroquine was performed at 100 ns to check the stability of protein. To turn these potential inhibitors into therapeutic medications, more in vivo and in vitro research is needed. The findings of this study are expected to be relevant in the development and exploration of innovative natural BSEP medicinal medicines in the future.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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