Virtual screening and molecular dynamics simulation studies to predict the binding of Sisymbrium irio L. derived phytochemicals against Staphylococcus aureus dihydrofolate reductase (DHFR)

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How to Cite

Abstract
The discovery of antibiotics initiated the era of drug innovation and implementation for human and animal health. Very soon, antibiotic resistance started evolving due to over-prescription and heavy usage of drugs leading to deleterious side effects. However, using plant extracts or medicinal plants has emerged as a new approach to dealing with the current problem. One such medicinal plant Sisymbrium irio L. is widely used in Unani therapy as an antimicrobial, analgesic, antipyretic, antioxidant, anti-inflammatory, hepatoprotective, bronchoprotective etc. The phytochemicals extracted from the aerial part of the plant have been used as a natural compound library and screened against a well-known anti-bacterial drug target Dihydrofolate reductase (DHFR) enzyme of Staphylococcus aureus. The top two phytochemicals with lower docking score along with the positive control were subjected to molecular dynamics (MD) simulation studies to examine the stabilities of the complexes over 100 ns, followed by binding free energy estimation. The Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Radius of Gyration (Rg) yielded established results throughout the MD run. Moreover, the derived phytochemicals exhibited lower binding free energy values than the positive control that can be tested for its in vitro efficacy, followed by further optimization to attain a potent therapeutic against S. aureus. Taken together, the present study suggests two promising phytochemicals derived from the aerial part of the plant S. irio with stable MD simulation results, strong binding affinity and no side effects.

Keywords: Binding free energy, DHFR, Phytochemicals, Sisymbrium irio L.

INTRODUCTION

In recent years, commercially used antibiotics have decreased in their actions due to the emergence of resistance in pathogenic bacteria (Adwan et al., 2010). Therefore, the researchers focus on the plants and their phyto-consituents having antimicrobial properties (Al Akeel et al., 2014). These phytochemicals may act on DNA replication, protein synthesis and cell wall synthesis during the division cycle of the bacterial cell (Alves et al., 2014). One of the medicinal plants Sisymbrium irio L. commonly known as London Rocket of the family ‘Brassicaceae’ is distributed in different parts of the world (Khosshoo, 1966). It is an annual winter herb, and the height of the plant is around three feet, with has open thin stem branches with pale yellow flowers (Ray et al., 2005). The aerial parts of the plant are being used in traditional medicine to treat cough, rheumatism, inflammation, chest congestion, swelling and cleaning wounds (Lev, 2003). The seeds of the plant are used as an expectorant and in treating voice disorders (Shah et al., 2014). Different studies revealed that the S. irio...
species contain various metabolites such as alkaloids, steroids, flavonoids, anthraquinones and fatty acids (Al-Jaber, 2011; Al-Qudah and Abu Zarga, 2010 a; Vahora et al., 1980). Many phytochemicals have been isolated through different studies from various parts of S. irdo such as alkaloids, tannin, saponins, flavonoids, glycosides, phenolics, glucosinolates, carbohydrates, fatty acids, amino acids, proteins and steroids that contribute to various pharmacological actions such as antibacterial, antifungal, anti-inflammatory, anticancer, analgesic, antiinflammatory, hepatoprotective and broncho-protective (Hailu et al., 2019). Studies showed that the aerial parts of S. irdo exhibit significant antibacterial activities against gram-positive and negative bacterial strains (Shabnam et al., 2015; Al-Massarani et al., 2017). Hence, with the aim to identify the probable site of action of natural antibacterial compounds in conjunction with their efficacy, the extracted phytochemicals from different parts of S. irdo, have been used as a screening dataset against a well known bacterial drug target. Molecular docking is a widely used technique to help researchers to predict the arrangement of small molecules within the 3D structure of the receptor along with the affinity of the ligand to the target with appreciable accuracy and efficacy (Kroemer, 2007 and Kumalo et al., 2015). The availability of protein molecule’s three-dimensional (3D) structure and high-end computation facilities has made it more feasible than ever before. The well-known antibacterial drug target used in the present study is DHFR, isolated from Staphylococcus aureus, PDB ID: 3SRW. DHFR is an essential enzyme, involved in catalysing the transfer of a hydride ion (H) from nicotinamide adenine dinucleotide phosphate (NADPH) to 7,8 dihydrofolate (DHF), results in forming 5,6,7,8 tetrahydrofolate (THF) as a product. It is the principle enzymes of THF pathway, involved in maintain the pools of THF and its derivatives inside the cell. THF and its derivatives act as an essential cofactors in many single-carbon transfer reactions such as biosynthesis of purine nucleotides, thymidylate, panthenohenate and other metabolites. Inhibition of DHFR blocks DNA replication and cell division, resulting in cell death (Oefner et al., 2009). The amino-acid residues present in the active site of DHFR are as follows Asp28, Leu29, Val32, Leu55, Ile57 and Phe63. DHFR is also a famous target enzyme against antibacterial, antifungal, antiparasitic and anticancerous agents and Trimethoprim is one of the most popularly used antibiotic against it. However, with the advent of time, both gram-positive and gram-negative bacteria have developed resistance against trimethoprim (Li et al., 2011). Therefore, there is a urgent need to search for suitable replacements for this conventionally used antibiotic with maximum efficacy and minimal side effect. Techniques like molecular docking and virtual screening are routinely used to unveil the binding potential for respective conformations of small molecules against the receptor. However, the freely available tools like: AutoDock4 and AutoDock Vina used for molecular docking fails to segregate the false positive and negative controls, hence, post-processing by the incorporation of MD simulation of the docked complexes is always for accurate docked orientation and ranking the top hits based on estimated binding free energies ($\Delta G_{bind}$) (Gupta et al., 2018). Among the various popular methods, molecular mechanics Poisson–Boltzmann surface area (MMPBSA) and molecular mechanics generalized born surface area (MMGBSA) are widely used for predicting binding affinities of receptor-ligand complexes (Massova and Kollman, 2000), as the above two techniques favours well-converged systems of receptor-ligand complexes generated during molecular dynamics (MD) simulation. Therefore, ‘Gromacs’ an open-source MD simulation package has been deployed to generate equilibrated trajectories of water-soaked receptor-ligand complexes (Pronk et al., 2013). On the above simulated well-stabilized trajectories, binding free energy calculation was performed using ‘g_mmpbsa’ tool to estimate the affinity between receptor (DHFR) and phytochemicals. The availability of 3D-structure renders feasibility to the SBDD (Structure-based drug designing) approach to discover novel antimicrobial compounds.

The present study aimed to identify potential candidates against antibacterial drug target, i.e. S. aureus DHFR (saDHFR), using nature derived phytochemicals from S. irdo. Virtual screening and molecular dynamics simulation (using gromacs) were performed to estimate the binding affinity of S. irdo derived phytochemicals against S. aureus DHFR.

**MATERIALS AND METHODS**

**Library preparation**

Over a few decades, molecular docking has gained rapid acceleration in the field of computational drug discovery. It involves the estimation of binding poses and energies corresponding to the generated conformations. The phytochemicals identified from the aerial parts of S. irdo were used to prepare virtual library and screened against well-known antibacterial drug target DHFR from S. aureus (PDB ID: 3SRW). Total 79 compounds (phytochemicals) identified in different studies (Khan et al., 1991; Griffiths et al., 2001; Al-Qudah and Abu Zarga, 2010 a and b; Al-Jaber, 2011; Alsaffar et al., 2016 and Al-Massarani et al., 2017) were used as an initial dataset. The smile structures of all the compounds were copied from the Pubchem database (Kim et al., 2016) and pasted on Molinspiration software to find out their drug likeness. Finally, 29 phytochemicals were filtered through Molinspiration software and found to follow all the rules of lipsinki rule of five and thus se-
lected for further analysis. Table 1 summarizes the list of 29 phytochemicals with Pubchem CID, along with the acceptable molecular weight values, no hydrogen bonds acceptor (nON), no Hydrogen bonds donor (nOHNH) and partition coefficient, required for a compound to act as drug. The compounds were downloaded in 3D sdf format and further converted to mol2 using Open Babel tool (O’Boyle et al., 2011). The well-known drug against bacterial DHFR i.e. Trimethoprim was taken as a positive control. Further, an inbuilt script 'prepare_ligand.py' of MGL Tool was used for batch conversion of ligands from mol2 format to Autodock Vina’s compatible format (pdbqt) to perform virtual screening (Morris et al., 2009; Trot and Olson, 2010).

Receptor preparation

The 3-dimentional crystal structure of DHFR (PDB-ID 3SRW) was downloaded from RCSB database. 3SRW is a co-crystal structure with NADPH and a ligand bound at active sites. NADPH was kept intact throughout the screening. For molecular docking using Autodock Vina, the receptor too needs to be present in pdbqt format, which was achieved using 'prepare_receptor.py' script incorporated in MGL Tools (Morris et al., 2009).

Molecular docking

As, the PDB-3SRW is a co-crystal structure, its active site is well characterised, yet to get the coordinates (grid centre; x,y,z) lying in the active site for molecular docking. Putative Active Site with Spheres (PASS) package was employed that characterized regions of buried volume to identify binding sites based on shape, size and burial extent of volumes (Brady and Stouten, 2000). The molecular docking was performed using Autodock Vina software with the grid size of x, y, z=25 and run number of 10. Pymol was used to visualise the bound confirmation of small molecules to the receptors (DeLano, 2002). The interactions of the docked receptor-ligand complexes (H-bond & hydrophobic interactions) were analysed using Ligplot+ program (Laskowski and Swindells, 2011). Out of multiple conformations generated by AutoDock Vina, the one with the least energy was selected for post-processing.

Molecular dynamics simulations

An all-atom MD simulation was performed on the receptor-small-molecules (Trimethoprim, CID_5280443 and CID_5280863) complexes using GROMACS 5.5.1 (http://www.gromacs.org/) via TIP3P water model (Van Der Spoel et al., 2005). The ‘pdb2gmx’ program was used to allocate missing force-field parameters to the receptor that parameterised using ff99SB-ILDN force-field (Lindorff-Larsen et al., 2010). The molecular mechanics parameters of all three ligands and NADPH molecule have been assigned using the ‘antechamber’ module of AMBER18 using the AM1-BCC charge method (Jakalian et al., 2002), and generalized amber force-field (gaff) (Wang et al., 2004). The AMBER parameter database was used to obtain the parameters for NADPH (http://research.bmh.manchester.ac.uk/ bryce/amber/) and further converted to Gromacs compatible format using a python script ‘Acyppe.py’ (Sousa da silva and Vranken, 2012). After that, the receptor-ligand complex was immersed in a 1.0 nm thick cubic box. The ‘editconf’ tool was used to apply PBC conditions by the addition of SPC water molecules to all the three systems. Since, all three ligands possess zero net charge, the ‘genion’ tool added 9 Na’ ions to neutralize the three different systems successfully. It was first energy minimized, to remove the bad contacts from the above systems, followed by a short NVT simulation (100 ps at 300K temperature). Subsequently, another 100 ps of simulation, at NPT ensemble (atmospheric pressure; 1 bar) along with position restraints on the macromolecule was performed to equilibrate the system. Once ensured that the systems were well equilibrated, they were subjected to a final production run for 100 ns, with time-step (2 fs). The ‘Parrninello-Rahman’ and ‘V-rescale’ thermostat algorithm was deployed as pressure and temperature coupling methods. During production phase, a number of vital parameters such as root-mean-square deviation (RMSD), root-mean square fluctuation (RMSF), radius of gyration (Rg) and solvent accessible surface area (SASA) were calculated from the trajectory (written every 10 ps) to investigate the dynamic stability of the receptor-ligand complexes. The principal component analysis (PCA) or essential dynamics (ED) of the MD trajectories was also performed to lessen the complication of the MD simulation coordinates to recognize the most significant motions. The eigenvalues and eigenvectors attained by diagonalizing the covariance matrix and the carbon-alpha motions of the two principal components (PC1 and PC2) were further inspected by the ED method. The ‘Qtgrace’, a plotting tool, generates all the graphs (https://sourceforge.net/projects/qtgrace/). The binding efficacy of each compound towards the DHFR receptor was assessed by the computation of binding free energy (ΔGbind) via MMPBSA approach with the help of the g_mmpbsa tool (Kumari et al., 2014).

Binding free energy estimation

Incorporated with MD simulation, binding free-energy calculation methods have emerged as robust mechanisms in bestowing quantifiable estimation for protein–protein and protein–ligand interactions. It is a crucial tool to explore the dynamic nature of the ligand inside the receptor cavity. A Gromacs compatible tool, ‘g_mmpbsa’ was used to compute the binding free energy (ΔGbind) between the receptor (DHFR enzyme) and the ligand of well equilibrated complex trajectories.
Here, all the energy components were calculated on 70 to 100 ns data at the time interval of 500 ps.

**RESULTS AND DISCUSSION**

Current research work is focused on predicting the inhibitory potential of the phytochemicals derived from aerial parts of *S. irio* against saDHFR enzyme. In the present study in-silico technique such as molecular docking was performed to predict the binding affinity and conformations of the small molecules to the above receptor. Detailed molecular docking analysis of the interactions between the receptor and the ligands has given some valuable compounds possessing the lowest binding scores compared to the positive control, which formed the base for selecting the top hits. Later, the behaviour of top molecules and positive control are studied in a dynamic state using the molecular dynamics approach.

**Molecular docking analysis**

Molecular docking is a widely used computational method to predict the mode of binding of chemical compounds to any specific receptor. The docking score and the molecules’ conformation decide the compounds’ affinity against the receptor. Fig.1 shows the binding conformations of the top five ligands in the active site pocket of the DHFR receptor. The NADPH molecule is bound at the site adjacent to the active site. Table 2 represents the top five compounds and the positive controls docked to DHFR receptor along with their AutoDock Vina score and residues interacting with the compounds. Compound CID_5280443, CID_5280863 and CID_5280343 had the docking score of -8.5, -8 and -7.9 kcal/mol respectively. These three compounds possess docking score even better than the positive control trimethoprim (-7.0 kcal/mol), though they possess lesser number of H-bonds. Compound CID_5280443 and CID_5280863 form 2 H-bond along with 8 and 6 lipophilic interactions, whereas CID_5280343 forms 1 H-bond and 7 lipophilic interactions. It has been observed here that more hydrophobic interactions correspond to lower binding score hence, strong affinity towards the receptor. Therefore, out of 29 phytochemicals, the compounds that exhibited lower binding scores than the trimethoprim, in conjunction with the conformational stability offered by the interactions among the receptor and the ligand via LigPlot+, laid the basis for selecting the top hits. The selected top hits and the positive control are subsequently promoted to MD simulation to estimate their conformational stability and the binding strength with the receptor.

**Molecular dynamics simulations**

Molecular dynamics simulation analysis has served as an excellent approach to understanding the behaviour of receptor and ligand with respect to each other in bound form. Here, we performed the explicit receptor-ligand complex simulations to evaluate the stabilities of the small-molecules at the binding pocket of the receptor protein using the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), hydrogen bond (H-bond), solvent accessible surface area (SASA), principal component analysis (PCA) and binding free energy ($\Delta G_{bind}$) computation using ‘g_mmpbsa tool’.

The RMSD analysis is used to access the conformational stability of the receptor and small-molecule in bound complex form during the MD run. Technically, the RMSD computes the deviations in the protein backbone from the initial to the final conformation, stating that lesser deviations correspond to a more stable protein (Aier et al., 2016). In the present study, the RMSD analysis was performed for the protein receptor backbone (REC-BB), receptor-ligand complex (REC-LIG-COM), receptor-ligand-NADPH complex (REC-LIG-NADPH-COM) and only ligands for both the compounds as well as for trimethoprim. Fig. 2A, 2B, 2C and 2D represent the REC-BB (black), REC-LIG-COM (red) and REC-LIG-NADPH-COM (green) RMSD plot for trimethoprim, compound CID_5280443 and CID_5280863 respectively over 100 ns data. All three RMSD plots of trimethoprim overlap and converge around 70 ns to the deviation of ~0.1 nm. This suggests that the trimethoprim exhibits stability at the active site vicinity of the receptor and forms an established complex in the presence of NADPH. The REC-LIG-COM and REC-LIG-NADPH-COM graphs for compounds CID_5280443 and CID_5280863 are almost overlapping. However, a surge is observed for the REC-LIG-COM/RMSD profile of CID_5280443 and CID_5280863 that further settles around 60 ns, indicating that the docked conformation was not appropriate initially, but it eventually stabilizes at the binding site. Furthermore, compound CID_5280443 in complex form exhibits a little higher RMSD (0.2 nm) as compared to the backbone (0.1 nm), but both the plots demonstrate stable RMSD profile from 60 to 100 ns, whereas the RMSD profile of CID_5280863 remained steady for both the REC-BB (black) and REC-LIG-COM (red) (0.1 nm). Moreover, the RMSD profile only for ligands shows a well-converged graph for trimethoprim (around 0.5 nm), and CID_5280863 however, CID_5280863 exhibits a bit higher deviation from 0.5 nm to ~1.0 nm. Also, none of the ligands exhibits more than 0.1 nm deviation in their RMSD profile. The above analysis suggests that the concerned ligand remained stable in the binding pocket of the receptor. Therefore, it can be inferred that MD simulation studies up-scales the results of any docking process that allows the small molecules to properly align into the binding cavity of the receptors, in a manner that promotes stabilized energy conformation.
The extent to form polar interactions between small molecules and receptor was investigated by identifying the number of hydrogen bonds (H-bonds) between the receptor and ligand during the simulation Fig.3. From the plot, it can be inferred that the trimethoprim possesses maximum number of H-bonds with the receptor throughout the 100 ns simulation, whereas CID_5280863 and CID_5280443 forms a significant number of H-bond interactions however, higher occupancy is observed for the former one. Thus, the above results suggest that the aforementioned phytochemicals can be considered as a promising candidate against *S. aureus* DHFR enzyme. The RMSF was monitored throughout the simulation to

<table>
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<tr>
<th>Phytochemicals</th>
<th>PubChem CID</th>
<th>Molecular weight</th>
<th>nON</th>
<th>nOHNH</th>
<th>mi LogP</th>
<th>References</th>
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<td>Quercetin</td>
<td>5280343</td>
<td>302.24</td>
<td>7</td>
<td>5</td>
<td>1.68</td>
<td>Khan <em>et al</em>., 1991</td>
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<tr>
<td>Isorhamanetin</td>
<td>5281654</td>
<td>316.26</td>
<td>7</td>
<td>4</td>
<td>1.99</td>
<td>Khan <em>et al</em>., 1991</td>
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<td>N-(n-propyl) acetamide</td>
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<td>2</td>
<td>1</td>
<td>0.34</td>
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<td>75263</td>
<td>101.17</td>
<td>1</td>
<td>0</td>
<td>2.28</td>
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<td>1</td>
<td>0</td>
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<td>3.04</td>
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<td>Apigenin</td>
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<td>270.24</td>
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<td>3</td>
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<td>Adenosine</td>
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<td>Al-Jaber, 2011</td>
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<td>1.09</td>
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<td>1,5,8-Trimethyl-1,2-dihydronaphthalene</td>
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<td>68057</td>
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<td>o-Benzyl-L-serine</td>
<td>78457</td>
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<td>12127</td>
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<td>5363898</td>
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<td>3.19</td>
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<td>but-3-en-2-one</td>
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<td>200.24</td>
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<td>0</td>
<td>2.42</td>
<td>Al-Qudah and Abu Zarga, 2010 b</td>
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</table>

\*nON= no of Hydrogen bonds acceptor, nOHNH= no of Hydrogen bonds donor
understand the fluctuation of each amino acid during the MD run. The RMSF profile of the protein for the three independent systems: trimethoprim (black), CID_5280863 (red) and CID_5280443 (green) are shown in Fig. 4A. As observed from the RMSF plots, higher fluctuations are observed for following amino acids 67TSFNVE72, F119, F129, K141 and E144 for all the three systems. Furthermore, mapping the aforementioned amino acids on the structure file revealed that these amino acids are primarily part of the loops

Table 2. Top five compounds and the positive controls docked to DHFR receptor along with their AutoDock Vina score, number of interactions and residues interacting with the compounds

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>Structure</th>
<th>Autodock Vina score (kcal/mol)</th>
<th>Number of H-bonds and residues involved</th>
<th>Number of lipophilic interactions and residues involved</th>
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<td>CID_5280443</td>
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<td>-8.5</td>
<td>2; Gln20, Asp28</td>
<td>8; Leu6, Val7, Ala8, Leu21, Leu29, Ser50, Ile51, Phe93</td>
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<td>CID_5280863</td>
<td><img src="image" alt="CID_5280863" /></td>
<td>-8</td>
<td>2; Gln20, Asp28</td>
<td>6; Val7, Leu21, Leu29, Ser50, Ile51, Phe93</td>
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<tr>
<td>CID_5280343</td>
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<td>-7.9</td>
<td>1; Gln20</td>
<td>7; Leu6, Val7, Ala8, Leu21, Asp28, Ser50, Ile51, Phe93</td>
</tr>
<tr>
<td>CID_5578</td>
<td><img src="image" alt="CID_5578" /></td>
<td>-7</td>
<td>3; Leu6, Asp28, Phe93</td>
<td>5; Val7, Ala8, Leu21, Leu29, Val32</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td><img src="image" alt="Trimethoprim" /></td>
<td>-7</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CID_68057</td>
<td><img src="image" alt="CID_68057" /></td>
<td>-7</td>
<td>-</td>
<td>8; Leu21, Asp28, Leu29, Val32, Thr47, Ile51, Leu55, Phe93</td>
</tr>
</tbody>
</table>

Fig.1. Binding conformations of the top five ligands in the active site pocket of the DHFR receptor
and reside way off from the active site; hence, little higher fluctuations will not influence the binding of small molecule to the receptor. These results infer that the presence of compounds has not caused any major fluctuation in amino acid residues of the enzyme during the simulation.

Rg is a measure of the compactness of the protein (Lobanov et al., 2008). Fig.4B depicts the measured Rg for the backbone atoms for all three systems trimethoprim (black), CID_5280863 (red) and CID_5280443 (green). The Rg plots for the above systems remained steady throughout the simulation run, representing the

<table>
<thead>
<tr>
<th>Compound</th>
<th>van der Waal energy (kJ/mol ± Std.dev)</th>
<th>Electrostatic energy (kJ/mol ± Std.dev)</th>
<th>Polar solvation energy (kJ/mol ± Std.dev)</th>
<th>SASA energy (kJ/mol ± Std.dev)</th>
<th>Binding energy: ΔG_{bind} (kJ/mol ± Std.dev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim (positive control)</td>
<td>-101.256 ± 1.104</td>
<td>-15.962 ± 1.196</td>
<td>72.963 ± 1.252</td>
<td>-16.578 ± 0.096</td>
<td>-60.609 ± 1.221</td>
</tr>
<tr>
<td>CID_5280443</td>
<td>-98.451 ± 1.421</td>
<td>-22.734 ± 1.166</td>
<td>63.979 ± 1.826</td>
<td>-12.125 ± 0.133</td>
<td>-69.209 ± 1.478</td>
</tr>
</tbody>
</table>
high compactness of the protein in all three systems portraying that the compounds did not hamper the native structure of the enzyme. The SASA value (Fig. 4C) is in the range of ~93nm² for all three systems, deciphering the presence of phytochemicals (CID_5280863 and CID_5280443) in the active site cavity of the receptor does not allocate any major structural change in the receptor’s geometry. The above analysis confirms that the trimethoprim and the phytochemicals do not perturb the configuration of the enzyme and the stability is conserved close to its native form. Overall, the RMSD profile of all three molecules (CID_5280443, CID_5280863 and trimethoprim) exhibits stability around 70 ns. Hence initial 70 ns data will not be considered for binding free energy estimation.

The essential subset gives the maximum protein dynamics that can be recognised with eigenvectors which are mostly associated with the eigenvalues. The dynamic behaviour of the DHFR receptor in the presence of trimethoprim (Fig. 5A) and the proposed phytochemicals (CID_5280863 and CID_5280443) (Fig. 5B & 5C) is illustrated in the form of PC1 vs. PC2 2D projection plots. The results revealed that the clusters representing the Cα-atoms of the receptor from three different systems are clearly separated, indicating the stability of the receptor in the presence of phytochemicals.
systems are well-defined by covering the minimum regions. However, the differences in the cluster size and PCs rearrangements were majorly due to the change in the structure of the ligands. Therefore, ligand binding does not generate any novel motions in the structure, but rather rearranges motions energetically in the dynamic range of the structure.

**Binding free energy estimation**

Additionally, to estimate the strength of binding of the above compounds to the receptor, g_mmpbsa tool has been deployed. The $\Delta G_{\text{bind}}$ and the contribution of individual components for both the ligands and the positive control are tabulated in Table 3. The $\Delta G_{\text{bind}}$ = kJ/mol for -69.209 CID_5280863, -74.928 kJ/mol for CID_5280443 and -60.609kJ/mol for trimethoprim, respectively.

S. aureus and other bacterial infections are the primary concern to public health due to the development of resistance against antibiotics. Many efforts have been made to develop a suitable replacement for commercially used antibiotics against S. aureus. One such study by Bourne et al. (2010) where a small molecule RAB1 (co-crystal Bacillus anthracis DHFR inhibitor) was found to be effective in binding the saDHFR as well. In another independent study, a three-step screening was performed on the saDHFR crystal structure using a chemical compound library. This study revealed five compounds (KBS1-KBS5) based on docking and simulations results, out of which, three compounds (KBS1, KBS3 and KBS4) exhibited in-vitro efficacy against wild-type as well as mutated saDHFR (Kobayashi et al., 2014). A recent in-silico study revealed two lead molecules exhibiting higher affinity towards saDHFR validated via MMGBSA method (Singh et al., 2022). Understandably, various efforts have been underway to find alternative antibiotics against S. aureus, but significantly less contribute to medicinal plant products.

Although many studies have already been done on the antimicrobial activities from aerial parts of S. irio against both gram-positive and gram-negative bacteria (Shabnam et al., 2015; Al-Massarani et al., 2017; El-Sherbiny et al., 2017), but none of the studies were done for the exploration of most potential phytochemical from S. irio responsible for the antibacterial property and its mechanism of antibacterial action. Therefore, the present research deciphers the virtual-screening of 29 selected phytochemicals from S. irio against saDHFR. The results confer that 3 phytochemicals show better Vina score with the receptor than the positive control (Trimethoprim). Out of three, the top two best-scored phytochemicals CID-5280863 (Kaempferol) and CID-5280443 (Apigenin) were further validated using MD simulation studies. The MD simulation studies have shown stable binding confirmations throughout the run. The affinity of both the phytochemicals for the DHFR receptor of S. aureus is higher than the positive control. The binding energies are also comparatively good compared to the most recent study (Singh et al., 2022). Moreover, previous studies exhibit the presence of a high percentage of both flavonoids (Kaempferol and Apigenin) in the aerial parts of S. irio (Al-Jaber, 2011), which shows the importance of the plant as the source of antibacterial agents and also gives the scientific proof to the folkloric claim of the plant. The above results prove that the compounds are the suitable binder of the antibacterial drug target DHFR, and can act as the right candidate that can later be optimised to tackle the resistance against antibiotics.

**Conclusion**

To address the problem of multi-drug resistance, a series of phytochemicals derived from the aerial parts of the plant S. irio, along with positive-control trimethoprim...
thoprim, screened against the active-site cleft of the DHFR receptor of S. aureus showed that phytochemicals CID_5280443 and CID_5280863 ranked at the top and exhibited lower docking scores compared to trimethoprim. The detailed analysis of their effect on the enzyme was studied using the MD simulation approach and its in-built analysis modules: RMSD, RMSF, H-bond, SASA, Rg and PCA. Both the investigated phytochemicals were found to have comparable RMSD profile, RMSF and Rg to the positive control however, the binding free energy values indicate a significant difference. Both phytochemicals also made a significant number of Hydrogen bonds with the receptor. These results showed that among various phytochemicals, CID_5280863 and CID_5280443 appeared to be strong binders which can further be optimized to attain potential therapeutics against S. aureus infections with minimal chance of side effects. Thus, it can be concluded that S. ino is a powerful candidate for discovering bioactive compounds possessing antimicrobial activities and may also serve for the development of novel pharmaceuticals.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


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