INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 11, Issue 3, 2023

ISSN - 2321-4406

Research Article

MOLECULAR DOCKING STUDY: TARGETING SICKLE CELL ANEMIA USING ACTIVE PHYTOCHEMICAL COMPOUNDS FROM ZANTHOXYLUM ZANTHOXYLOIDES

MOULI DAS*

Department of Biotechnology, Government City College, Hyderabad, Telangana, India. Email: moulidas292001@gmail.com

Received: 30 March 2023, Revised and Accepted: 28 April 2023

ABSTRACT

Objectives: A category of genetic disease which is sickle cell disease is called sickle cell anemia. In this, the red blood cells are affected in shape and structure, which normally carries oxygen to every part of our body. Therefore, by using molecular docking studies, Absorption, Distribution, Metabolism, and Excretion (ADME) analysis for phytocompounds, an effort was made to find natural compounds from a plant named *Zanthoxylum zanthoxyloides*, which has many medicinal properties in Indian Ayurveda, to prevent this genetic disease.

Methods: The Protein Data Bank database was used to retrieve the primary protein, hemoglobin. The PyRx tool was used to perform docking because the ligands were poorly binding with the compounds and were interfering with the docking, so this tool was used. Swiss-ADME and the Admetlab web server were used for the analysis of ADME and drug similarity.

Results: Five chemicals from *Z. zanthoxyloides* have been identified through molecular docking investigations as having high binding affinity to the protein by inhibiting the replication of viruses and proteolytic cleavage. Out of these five compounds, Benz[c]acridine, Fagaramide trans-fagaramide was safe and possessed drug-like qualities according to the ADMET profile and drug similarity prediction.

Conclusion: According to the current study, Benz[c]acridine and Fagaramide trans-fagaramide have a particular binding affinity and aid in the management of treatment approaches for treating sickle cell anemia.

Keywords: Sickle cell anemia, *Zanthoxylum zanthoxyloides*, Red blood cell.

 DOI: http://dx.doi.org/10.22159/ijms.2023v11i3.47983. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

Sickle cell anemia causes a hereditary illness called sickle cell disease, in this the structure of the red blood cells (RBCs) becomes crescent-shaped which cannot transport oxygen properly to our body. Normally, RBCs are spherically shaped and flexible, which allows them to travel through the blood channels. Some RBCs in sickle cell anemia have a sickle or crescent shape [1]. These sickle cells stiffen and cling together, delaying or stopping blood flow. This disease does not have cure for some people. Treatment can remove discomfort and help to avoid disease-related consequences. Sickle cells degrade and die quickly. Normal RBCs have life span of 120 days. However, the life span of sickle cell is about 10–20 days, which causes the shortage of RBCs (anemia). Fatigue arises when the body cannot acquire adequate oxygen due to a lack of RBCs [2]. The major symptom of sickle cell anemia is repeated, agonizing pain, which is frequently referred to as a pain crisis. RBCs with a crescent shape that restrict blood flow to the joints, chest, and abdomen cause irritation when they flow through narrow blood channels. The discomfort may linger for hours or days and vary in intensity. Only a few times a year, some people feel discomfort. Others get twelve or more per year. Hospitalization is necessary for severe pain episodes. Due to ulceration, bone and joint degeneration, and other conditions, some adults and adolescents with sickle cell anemia have persistent pain. It has been employed to address several pathological. Especially sickle cell anemia, one of the most prevalent hereditary blood diseases that plague people with African heritage. A bioactive *Zanthoxylum zanthoxyloide* with oxygen affinity-modulating action should be identified and evaluated [3]. Phytochemical components of *Zanthoxylum zanthoxyloides* (Lam.) stem bark were detected by gas chromatography-mass spectrometry. To test for in silico anti-sickling activity, Zepern and Timler were used. 7-(dimethylamino)-2,3-dihydro-1H-cyclopenta[c] Chromen-4-one and (8S,9S,10R, 13S,14S, 16R,17S)-17-acetyl-6,10,13,16-tetramethyl-8,9,11,12,14,15,16,17-octahydrocyclopenta[a] phenanthrene-3-one hemoglobin have significant affinity for the active site of the alpha globin subunit, which is located at (valine 1). Additional examination of the ligand binding locations showed the emergence of advantageous interactions, such as hydrogen bonds, cations, van der Waals, and hydrophobic contacts. The pharmacokinetic characteristics of 7- (dimethylamino) -2,3-dihydro-1H-cyclopenta[c] both Chromen-4-1 and (8S,9S,10R,13S,14S,16R,17S) 6,10,13,16-tetramethyl 8,9,11,12,14,15,16,17-octahydrocyclopenta[a] -17-acetyl. The profile of phenanthrene-3-one indicates that it has high oral bioavailability and is drug-like [3]. This outcome strengthens the case for zanthoxylum xantoxyloides' use in the management of sickle cell anemia. Traditional medicine of Africa has long employed this plant *Z. zanthoxyloides* to treat sickle cell anemia.

METHODS

Protein preparation

The 3D structure of sickle cell anemia hemoglobin was downloaded from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org/). Normal hemoglobin is composed of four globin chains, each containing an iron-containing heme molecule. Globins are complex proteins that contain precise amino acid sequences and can fold into complex conformational patterns [4]. Protein crystal structures were purified before start docking studies to optimize hydrogen bonding and eliminate atomic collisions. Protein purification was performed using the standard procedure of Discovery Studio Visualizer 21.1. After removing water molecules and heteroatoms from the protein structure, polar hydrogens were added. In addiition, a prediction of the active site of the protein produced was performed.

Ligand selection

A total of 82 active plant compounds from *Z. zanthoxyloides* were retrieved from various literatures for the documentation of potential

inhibitors of sickle cell anemia. From the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/), structures of Phytocompounds were obtained in 3D SDF (Three-Dimensional Structure Data File) format. Ligand fabrication was accomplished by optimization, energy minimization, and conversion of ligand molecules to its 3D PDB format using his PyRx program.

Molecular docking

Molecular docking approaches describe how tiny compounds react at target protein binding sites. and simulate fundamental biochemical processes at the atomic level of interactions between small molecules and proteins. can be better understood [5]. P For molecular docking studies, the PyRx Virtual Screening Tool program was employed [6]. All his 82 active plant compounds of *Z. zanthoxyloides* were docked into the hemoglobin protein using the PyRx tool. For docking studies, the prepared receptor and ligand files were selected and targeted. For docking, proteins were loaded and transformed into macromolecules. The ligands were then imported and prepared using the tool's open babel tab. After establishing the protein and ligand molecules, we maximized the grid box to investigate all possible ligand-protein interactions. Once everything was set up, clicking the forward button started the docking. After docking was completed, we received a table showing the binding affinities of the individual ligands. The top five ligands were chosen for further investigation based on their highest binding affinities. The top five selected docked models were then saved as PDB files. Discovery Studio Visualizer 21.1 was used to conduct 2D-3D interactive visualization experiments [7].

Absorption, distribution, metabolism, and excretion (ADME) analysis

In pharmacokinetics and pharmacology, the phrase ADME, which stands for "ADME," refers to how a medication is eliminated from an organism. A compound's performance and pharmacological activity as a drug is influenced by four criteria. All of these criteria influence drug levels and the kinetics of drug exposure to tissues. Toxicity may also be considered, the so-called ADMET [8]. In this study, the top 5 compounds with the highest binding affinities were further analyzed for drug similarity ADMET using SWISSADME [9] and ADMETLAB (https://admetmesh.scbdd.com/service/evaluation/index) [10]. Boiled-Egg analysis was also carried out using the SWISS ADME tool [11]. Lipinski's rule of five was considered in the ADME analysis.

a. Molecular mass should be <500 dalton
b. log P should be <4.15
c. H-bond donor count should be <5
d. H-bond acceptor count should be <10 and
e. Molar refractivity should be between 40 and 130.

RESULTS

Molecular docking

Molecular docking studies revealed that several active plant compounds of *Z. zanthoxyloides* possess significant binding affinities with hemoglobin proteins. Table 1 presents a list of his five most important plant compounds of *Z. zanthoxyloides* that have the highest binding affinities for hemoglobin proteins. In accordance with the results of molecular docking studies using PyRx, We discovered that 15 of the 82 compounds from *Z. zanthoxyloides* have significant binding affinities with hemoglobin proteins. From the docking results, we selected the top five compounds (Table 2) for drug-likeness prediction and ADME analysis.

Molecular visualization

Discovery Studio Visualizer 21.1 was used to visualize interactions between the receptor ligands of the five most significant phytochemical with the highest binding affinities. Using PyRx, docked ligands were saved in PDB format and opened with purified hemoglobin protein. Various 2D and 3D interactions between plant compounds and hemoglobin proteins have been observed. Van der Waals forces, conventional and carbon-hydrogen bonds, -sulfur contacts, alkyl and -alkyl interactions, T-shaped interactions, and unfavorable interactions are all illustrated in the 2D interaction diagram [12].

Drug-likeness prediction and ADMET analysis

Based on the five aspects, Lipinski's rule of five compounds determines whether the compound is drug-like or non-drug-like molecules. Lipinski's rule of five was used to perform drug similarity predictions for the best docking compounds and the Swiss-ADME web server and the ADMETLAB 2.0 were used for ADME analysis. Boiled eggs were also analyzed using the Swiss ADME tool. In addition, Swiss-ADME tools were used to perform analyzes using boiled eggs to predict blood–brain barrier (BBB) and human intestinal absorption (HIA) of selected plant compounds. In addition, optimally docked compounds have been shown to have their water solubility (LogS), HIA, BBB, permeable glycoprotein substrate, carcinogenic activity, and Lipinski effect [13].

DISCUSSION

Nowadays, the majority of sickle cell disease patients are treated with hydroxyurea and crizanlizumab, two VOC-prevention medications Targeted therapy based on the pathophysiologic pathways of sickle cell disease that cause organ malfunction and painful episodes include hydroxyurea, L-glutamine, crizanlizumab, and other medications that are now available or on the horizon. Natural phytocompounds derived from medicinal plants, such as *Z. zanthoxyloides*, can be employed since they are less hazardous than manufactured chemicals [14]. In silico approaches like as molecular docking, ADME analysis, and molecular dynamic simulation have been found to be useful for future study into the binding affinities, interactions, and stability of ligands with target proteins. The Ramachandran plot depicts the statistical distribution of the combinations of backbone dihedral angles ϕ and ψ. The current investigation revealed the involvement of phytocompounds from *Z. zanthoxyloides*, which has several therapeutic benefits according to Ayurveda. According to the study, several phytocompounds from *Z. zanthoxyloides* have been discovered to be useful against Sickle cell anemia. For the research study, hemoglobin protein (3BJ1) was examined for the Ramachandran plot to confirm the purity of the protein, and no outliers or weak rotamers were found. Z-Score of protein observed was –1.91 for whole residues using a molecular docking technique, the binding affinity of all 82 phytocompounds was tested against Hemoglobin protein (PDB ID: 3BJ1) for in silico investigation [15]. Among them, five phytocompounds from, *Z. zanthoxyloides* namely, Benz[c]acridine, Fagaramide trans-fagaramide, Tricin 5-glucoside,

Table 1: Absorption, distribution, metabolism, and excretion analysis of best docked compounds based on Lipinski's rule

Serial number	Ligand name	Molecular weight (g/mol)	H-bond donor	H-bond acceptor	LogP	Molar refractivity
1	Benz[c] acridine	229.28	0	1	2.50	76.76
2	Fagaramide trans-fagaramide	247.29	1	3	2.92	69.63
3	Tricin 5-glucoside	492.43	5	12	2.60	116.41
4	(6R,7R)-7-[[2-(1-benzofuran-2-yl) acetyl] amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2-carboxylic acid	240.75	0	1	0.00	74.79
5	Butibufen	220.31	1	2	3.07	67.43

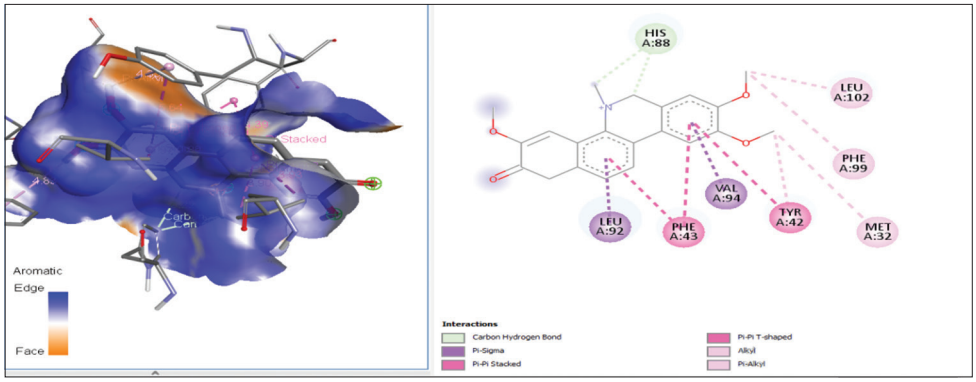

Fig. 1: 2D and 3D diagram of interaction of Benz[c]acridine with hemoglobin protein

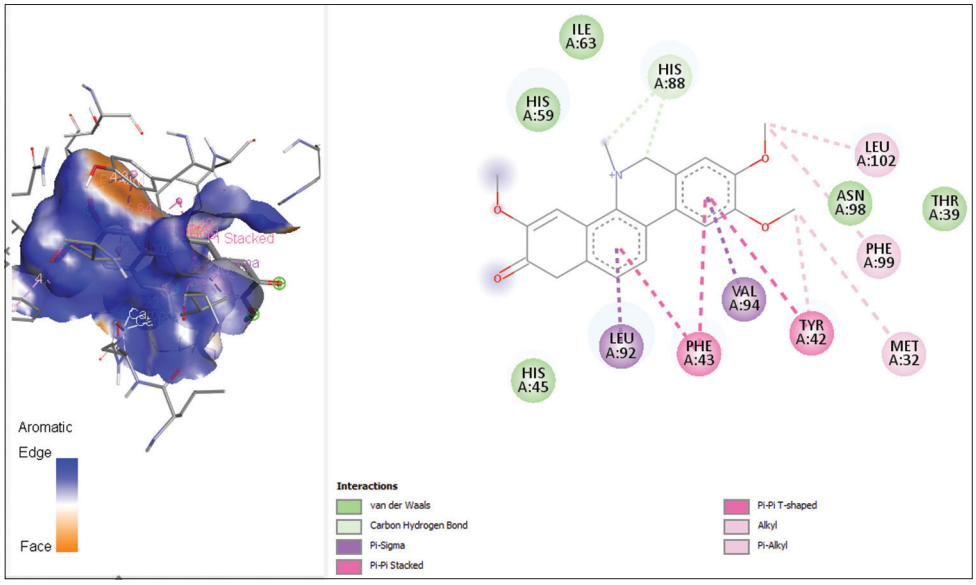

Fig. 2: 2D and 3D diagram of interaction of fagaramide trans-fagaramide with hemoglobin protein

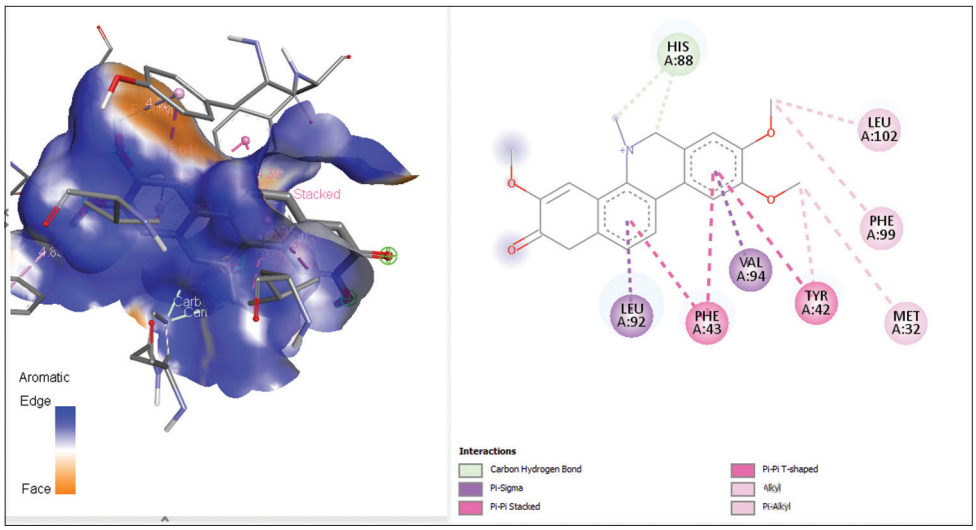

Fig. 3: 2D and 3D diagram of interaction of tricin 5-glucoside with hemoglobin protein

(6R,7R)-7-[[2-(1-benzofuran-2-yl)acetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, Butibufen shown significant binding affinity. After molecular docking study, all the five compounds were further studied for ADME analysis to validate the drug likeness and toxicity. The binding of these phytochemicals with Hemoglobin helps in cleavage of polyproteins to slow down the viral transcription and replication. Among these identified phytocompounds, Benz[c]acridine, Fagaramide trans-fagaramide based on their

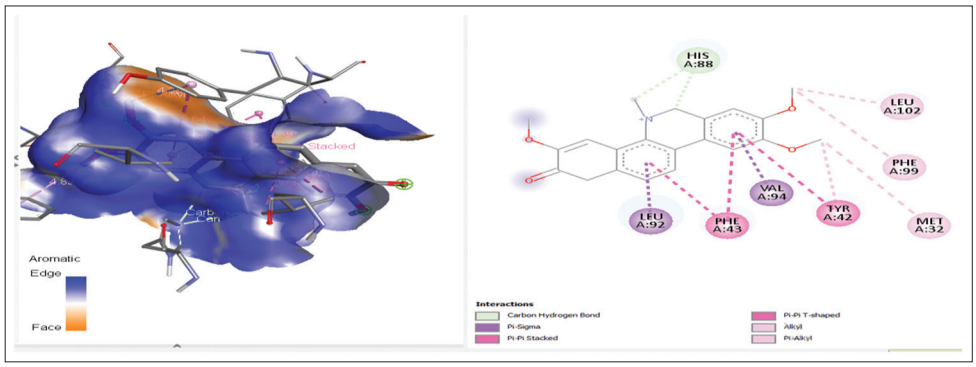

Fig. 4: 2D and 3D diagram of interaction of (6R,7R)-7-[[2-(1-benzofuran-2-yl)acetyl]amino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid with hemoglobin protein

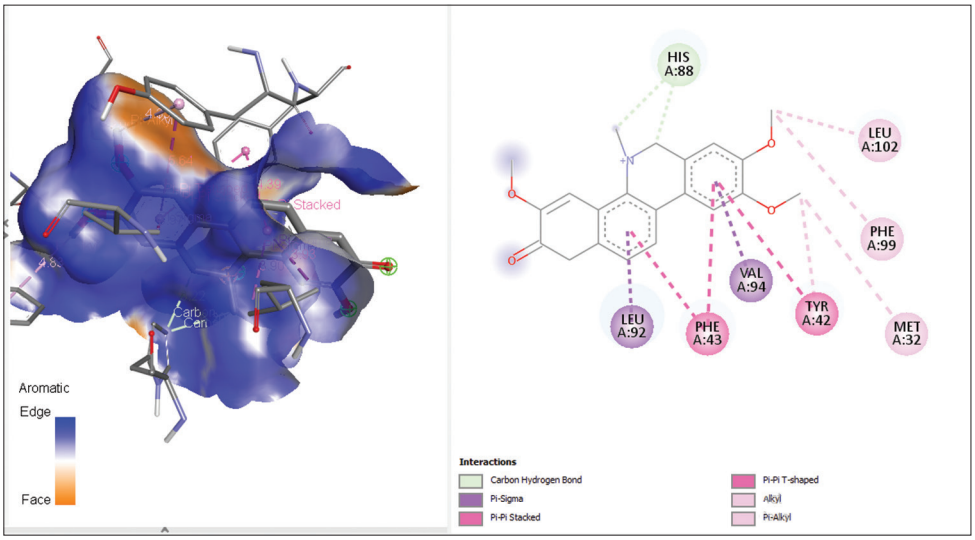

Fig. 5: 2D and 3D diagram of interaction of butibufen with hemoglobin protein

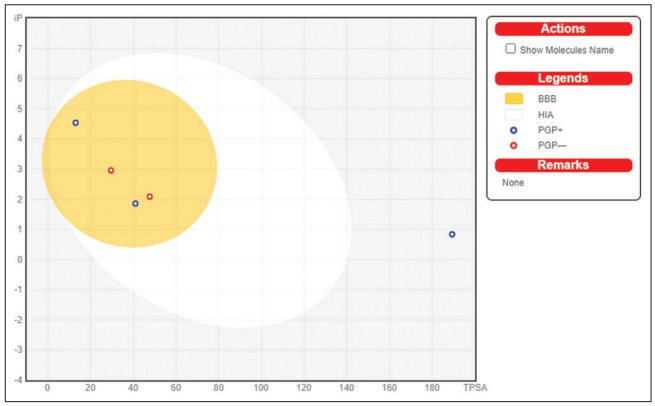

Fig. 6: Boiled egg-analysis of all the 5 top compounds

significant binding affinity, they can be predicted as potential inhibitors. These phytocompounds aid to block viral protein interactions with host cells and have been found to be safe and effective against Sickle cell anemia without causing toxicity.

CONCLUSION

The current study aims to identify natural phytocompounds from *Z. zanthoxyloides* that can serve as a treatment for Sickle Cell Anemia. It is a disease triggered by mutated hemoglobin protein which results in rigid and sickle shape of RBCs. RBC hemoglobin has significant potential and is a significant target for treating Sickle Cell Anemia [16]. Natural phytocompounds may block viral entry, preventing further replication and spread. We may conclude from this work that two phytocompounds from *Z. zanthoxyloides* are predicted to inhibit the function of mutant hemoglobin by blocking the translation of viral proteins, which aids in the damage to host cells. These phytocompounds have the highest potential inhibitory and hemoglobin binding affinity. With the use of ayurvedic treatments, the best-docked phytocompounds with drug-like characteristics, a safe ADMET profile, and efficacy may help to avoid Sickle cell anemia.

REFERENCES

1. Mangla A, Ehsan M, Agarwal N, Maruvada S. Sickle cell anemia. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2022.
2. Arkuszewski M, Krejza J, Chen R, Melhem ER. Sickle cell anemia: Reference values of cerebral blood flow determined by continuous arterial spin labeling MRI. Neuroradiol J 2013;26:191-200.
3. Okagu IU, Ndefo JC, Aham EC, Udenigwe CC. *Zanthoxylum* species: A review of traditional uses, phytochemistry and pharmacology in relation to cancer, infectious diseases and sickle cell anemia. Front Pharmacol 2021;12:713090.
4. Olushola-Siedoks AA, Igbo UE, Asieba G, Damola IA, Igwe CC. GC-MS aided *in silico* molecular docking studies on the anti-sickling properties of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler stem bark. J Pharmacogn Phytochem 2020;9:8-18.
5. WebPath. Available from: https://medicine.tamu.edu
6. McConkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. Curr Sci 2002;83:845-56.
7. Trott O, Olson AJ. Autodock vina improving the speed and accuracy of docking with a new scoring function, efficient optimization, and

multithreading. J Computat Chem 2010;31:455-61.
8. Wanarase S, Chavan S, Sharma S. A molecular docking study: Targeting covid-19 (SARS-CoV-2) main protease using active phytocompounds from *Terminalia arjuna*. Innov J Med Sci 2022;10:9-14.
9. Wikipedia Contributors. ADME. Wikipedia, The Free Encyclopedia; 2022. Available from: https://en.wikipedia.org/w/index.php?title=ADME&oldid=1087865915
10. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017;7:42717.
11. SwissADME. Swissadme.ch. Available from: http://www.swissadme.ch [Last accessed on 2023 Mar 16].
12. Drug-Likeness and Molecular Property Prediction. Molsoft.com. Available from: https://molsoft.com/mprop [Last accessed on 2023 Mar 16].
13. EMA. New Treatment for Sickle Cell Disease. European Medicines Agency; 2021. Available from: https://www.ema.europa.eu/en/news/new-treatment-sickle-cell-disease [Last accesseed on 2023 Mar 16].
14. Ahmed SF, Quadeer AA, Barton JP, McKay MR. Cross-serotypically conserved epitope recommendations for a universal T cell-based dengue vaccine. PLoS Negl Trop Dis 2020;14:e0008676.
15. Patil VM, Balasubramanian K, Masand N. Dengue virus polymerase. In: Viral Polymerases. Netherlands: Elsevier; 2019. p. 387-428.
16. Brewster B. Sickle cell anaemia: Causes, signs, symptoms and treatment. Nurs Times 2003;99:30-2.