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A MOLECULAR DOCKING APPROACH TO STUDY BINDING MOLECULAR INTERACTIONS BETWEEN HERBAL COMPOUND BALANITIN-6 AND *PLASMODIUM VIVEX* DIHYDROFOLATE REDUCTASE

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ABSTRACT

In this paper, herbal compound Balanitin-6 a diosgenyl saponin, found in the bark and seed of *Balanites aegyptiaca* has been investigated as potential inhibitor of *Plasmodium vivex* didydrofolate reductase (PvDHFR) activity. Molecular interaction analysis using Ligplot, Chimera, and PyMol of Balanitin-6/PvDHFR complex have revealed three hydrogen bonds with active site residues Ser58, Ser120, Arg131 and ten hydrophobic interactions in Site1 hydrophilic domin. The minimum binding energy was found to be -21.31Kcal/Mol and the inhibition constant (Ki) value of 237.79 aM which is 10^{12} times higher than other Food Drug Administration (FDA) approved drugs. The possible molecular interactions in close proximity to FDA approved drugs have been acknowledged and due to close proximity of Site1 active residues, where it forms part of the substrate-binding cleft, it is likely that displacement of Site1 region will interfere with substrate binding. Hence, Balanitin 6 targeted to Site1 might have sufficient profound effect to inhibit malarial protein PvDHFR activity.

KEYWORDS: **Malaria, *Plasmodium vivex* dihydrofolate reductase, molecular docking, Balanitin-6.**

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INTRODUCTION

Plants have been an integral part of human life in many tropical and subtropical countries including India. The plants have been widely used as antimalarials by traditional healers are significantly more active in vitro against *Plasmodium falciparum* and *Plasmodium vivax* than plants that are not widely used or not used at all, for the treatment of malaria[1]. This represents potential natural sources for the discovery of lead molecules for development into potential antimalarial drugs[2-4]. However, the WHO report states that although there is a widespread use of traditional herbal remedies in the supervision of malaria, scientific understanding of these plants is largely unexplored[5]. The major cause of disease and death from malaria in tropical and subtropical countries is due to the human parasite *P. falciparum* [6-7]. Spreading of diseases is associated with the development of a sexual parasite stages that undergo repeated cycles of invasion and replication in red blood cells (RBCs). A small proportion of mature gametocytes from differentiated parasite can undergo sexual development in the mosquito vector[8], which is responsible for the spread of the disease and death [9-10]. Antimalarial drugs are well known to block the action of dihydrofolate reductase (DHFR) which the parasite uses to protect them inside blood cells. DHFR plays a central role in promoting cell growth and proliferation, which is the main target for several anticancer and antibiotic drugs. Antimalarial drugs bind to DHFR protein and prevent its binding with the toxic haem and thus inhibit the growth of malaria parasite[11]. In malaria pharmacology, dihydrofolate reductase (DHFR) is of particular interest because traditional drugs such as pyrimethamine and cycloguanil are known to inhibit parasite[12]. At present, the available drugs used to fight the disease are quinine[13], antifolates-dihydrofolate reductase inhibitors, such as pyrimethamine[14] and artemisinin derivatives[15-17]. Nearly, every available therapeutic anti-malarial compounds encountered serious obstacles in treatment of malaria-endemic in countries like India, Pakistan, Africa and tropical and subtropical regions of the world due to developed at least partial resistance to date including the first-line drugs chloroquine and sulfadoxine-pyrimethamine[18-19]. A single-drug treatment is no longer sufficient, combinations of two or more drugs will probably offer improved efficacy and reduced risk of emergence of drug-resistant *P. vivax*. Current examples of drug combinations are artemisinin-amodiaquine and artemether-lumefantrine[20]. The lack of an effective vaccine and the generation of drug-resistant strains clearly indicate the need for the development of new therapeutic approaches and identification of novel targets.

Computer aided simulation of molecular interaction and prediction of possible active site moieties can be done using bioinformatics tools which help drug target prediction. Newer drugs are required to satisfy the numerous unmet clinical needs in many disease indications. *In-silico* virtual screening and computer-aided drug design have become increasingly important to design new active novel pharmacophores, and enzyme inhibitors that bind to the active sites of parasite protein and bocks their action[21]. In this paper, we have identified both qualitatively and quantitatively, natural pharmacophore molecules, based on DHFR interactions. The results were compared and the best inhibitor lead compound has been identified. The potential effective compounds predicted by this study can be further evaluated *in-vitro* and *in-vivo* biological assays and may help in future to derive new drug with higher potency and specificity with less or no side effect from natural sources.

MATERIALS AND METHODS

(i) Retrieval of PvDHFR from RCSB protein databank

The 3D structure of PvDHFR was downloaded from RSCB PDB with pdb code id: 2BL9 [22]. The bound ligand was removed from PDB file and the energy minimization was done before docking experiment by using Yet Another Scientific Artificial Reality Application (YASARA) [23].

(ii) Ligand Selection and Preparation

20 herbal drugs viz., 6(E)-Geranylgeraniol-19-oic acid, 9-Normethylbudmunchiamine K, allanxanthone C, alpha-Obacunyl acetate, balanitin 6, Benzoisoquinoline-5-10-dione, Budmunchiamine G, budmunchiamine K, Cassiaoccidentalin B, Cassiaoccidentalin C, catechol, cinnamolide-3beta-acetate, ethyl oxo(2-oxocyclohexyl)acetate, Exiguaflavanone B, isosungucine, oleanolic acid, scutianthraquinone C, tovophyllin, ursonic aicd, vernonioside A(1-4) and their 1500 derivatives were retrieved from NCBI PubChem (http://pubchem.ncbi.nlm.nih.gov), Chem Spider (http://www.chemspider.com), Chemical Register (http://www.chemicalregister.com/), Chemical Book (www.chemicalbook.com) and Chbi (www.chibi.ubc.ca) and were virtually screened on the basis of Lipinski's rule of five[24]. The ligands were converted into PDB coordinate files using OpenBabel software (www.openbabel.org) and were prepared by adding hydrogen atoms and neutralization of charged groups.

(iii) Molecular Docking

The energy minimized PvDHFR PDB file was generated by use of Swiss PDB viewer (www.SPDBV.vital-it.ch/). Kollman charges, polar hydrogen atoms and solvation parameters were added to PvDHFR structure after energy minimization step[25]. 3D grid maps for calculating atomic energy potentials for each atom in the ligand molecule which surround the binding site on the receptor molecule was generated using AutoGrid program available with AutoDock4.2. The grid map was created in such a way that the entire hydrophilic region of PvDHFR was covered. The box was set to 124Å×98Å×126Å with grid points separated by 0.375Å. Docking was performed and Lamarckian genetic algorithm was used to find the most preferred pose where the ligand can bind to the receptor with lowest binding energy. The results of docking studies were analyzed using LIGPLOT[26], PyMol[27] and UCSF Chimera[28] as per our previous studies as well.

(iv) Analysis and confirmation of Docking Results

The search for the best ways is to fit ligand, into PvDHFR structure, using Autodock4.2 which resulted in docking files that contain details including records of docking[29]. Auto Dock Tool (ADT) and Python scripts in MGL tools package were used to analyze the docking results summarized in log files. The similarity of docked structures was measured by computing the RMSD between the coordinates of the atoms and creating clustering of the conformations based on the RMSD values. The most favorable docking poses were identified on the basis of lowest binding energy conformations in all clusters.

RESULTS

In this study herbal compounds have been targeted to PvDHFR active site domains residues, using Autodock4.2 which plays critical role in the catalytic activity of PvDHFR. 1500 herbal compounds virtually screened for highest fit value and were subsequently analyzed for binding pattern to Site1 in PvDHFR. The active site residues were identified using CASTp and Q-Site finder and further verified through extensive literature search and by analysis of ligand and PvDHFR docked structures reported in RCSB databank. The residues Ile13, Phe206, Glu211, SERer120, Asp53, Phe36, Ile173, Tyr179, Thr194, Asn50, Met54, Phe57, Leu45 and Arg195 are conformed in PvDHFR for its enzymatic activity and binding molecular contacts of lignad on them may lead to the inhibition of PvDHFR activity[30-31].

(i) Docking of Balanitin 6 (CID: 44576182) to PvDHFR protein

Balanitin 6 was found bound in hydrophilic domain of PvDHFR with lowest minimum binding energy of -21.31Kcal/Mol which signifies greater affinity of molecular interactions which may lead inhibition of PvDHFR activity. The Ligplot, Chimera and PyMol analysis revealed Balanitin-6 was found bound into the targeted hydrophilic domain of PvDHFR with three residues Ser58, Ser120 and Arg131 in the vicinity of active Site1. Furthermore, molecular interaction analysis

revealed 10 hydrophobic bond interactions with Leu128, Tyr125, Ile121, Leu45, Gly43, Thr44, Gly40 and Val120 in PvDHFR Site1 (Fig. 1). The free binding energy (ΔG) of PvDHFR/Balanitin-6 complex was found -21.31Kcal/Mol which has been recorded highest among the all selected herbal compounds including the FDA approved drugs such as amodiaquine, chloroquine, proguanil, pyrimethamine, sulfadoxine and quinine (Table 1). Most importantly the inhibition constant (Ki) value was found 237.79 aM, which is 10^{12} times higher than other FDA drugs viz., quinine, chloroquine, pyrimethamine, amodiaquine (Fig. 3, Table 1), sulfonamide (Fig. 4, Table 1) and quinine (Fig. 5, Table 1) used to treat malaria, is an indication of how potent Balanitin-6 inhibitor is; it is also the concentration required to produce half maximum inhibition. Due to the close proximity of Site1 to the active site, where it forms part of the substrate-binding cleft, it is likely that displacement of Site 1 region will interfere with substrate binding and may lead to inhibition. Therefore, Balanitin 6 targeted to Site1 might have sufficient profound effect to inhibit malarial protein PvDHFR activity. Hence, it could be a promising potential drug candidate for malaria using PvDHFR Site1 as a Drug target and further in-vivo evolution would required to recommend it as a drug for malaria.

(ii) Docking of Isosungucine (CID: 5471853) to PvDHFR protein

The docking of isosungucine also have been performed on PvDHFR to study molecular interaction between isosungucine and PvDHFR. Isosungucine also found bound with targeted hydrophilic domain, with residue Ile173 in active Site1. Additionally, 12 hydrophobic interaction with residues Leu128, Met54, Gly174, Phe57, Cys14, Ser117, Ala15, Asp53, Leu45, Ile121, Phe122 and Tyr125 in PvDHFR site1 also identified using Ligplot (Fig. 2). The free binding energy (ΔG) of PvDHFR/Isosungucine complex was found -12.88 Kcal/Mol which is second highest with LogP value of 3.5 and an inhibition constant (Ki) value of 597.16 pM (Table 1). The hydrogen and hydrophobic bond interactions provides strong evidence for inhibition potential of isosungucine to PvDHFR activity. Due to the close proximity of Site1 active site, again it is likely that displacement of Site1 region will interfere with substrate binding and may also lead to inhibition of PvDHFR. Therefore, isosungucine targeted to Site1 will also have profound effect to inhibit malarial protein PvDHFR.

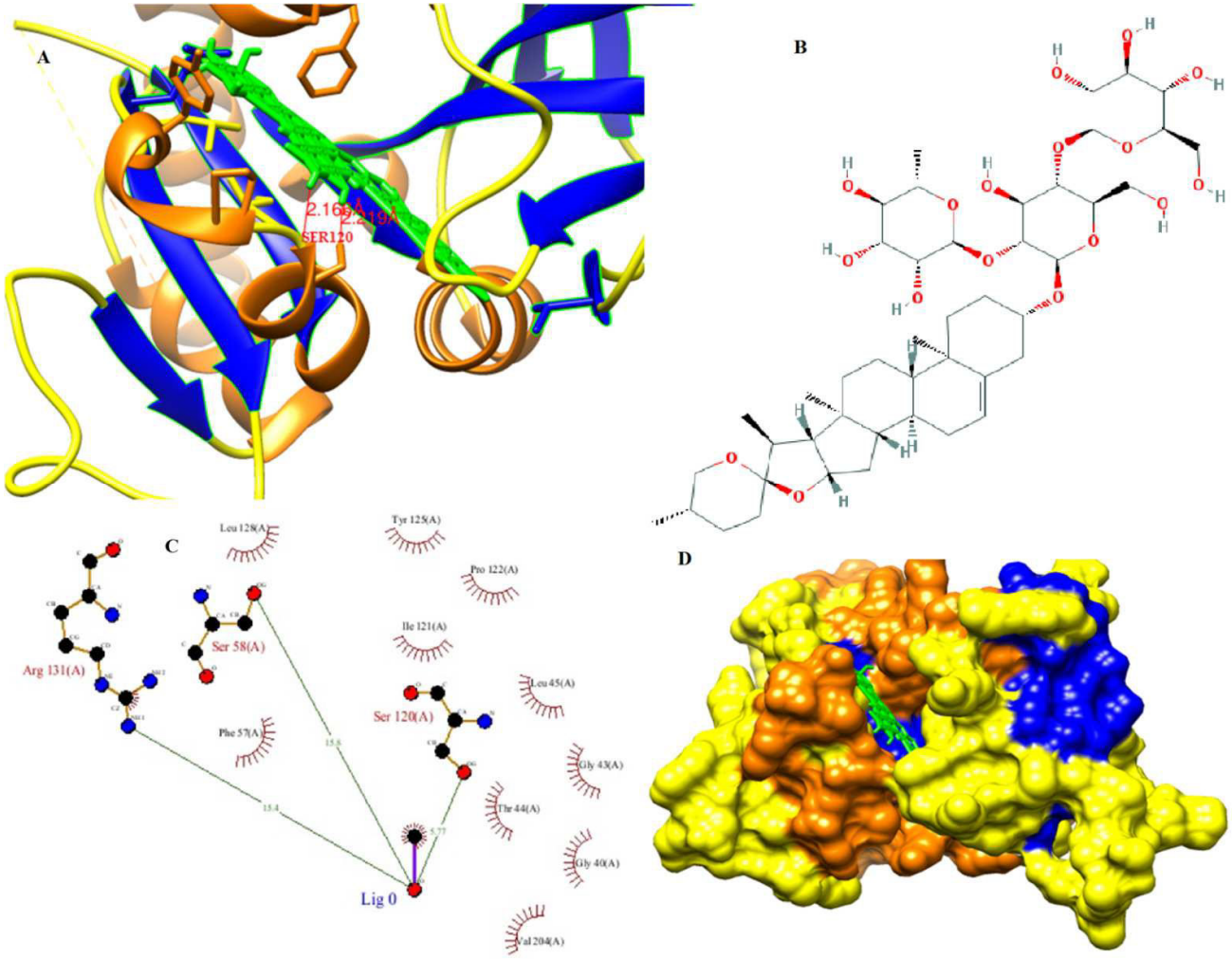

Figure 1
A - Docked Balanitesin-6 on PvDHFR, B- Chemical structure of Balanitesin-6, C- Ligplot and D- Surface image of PvDHFR cavity with ligand

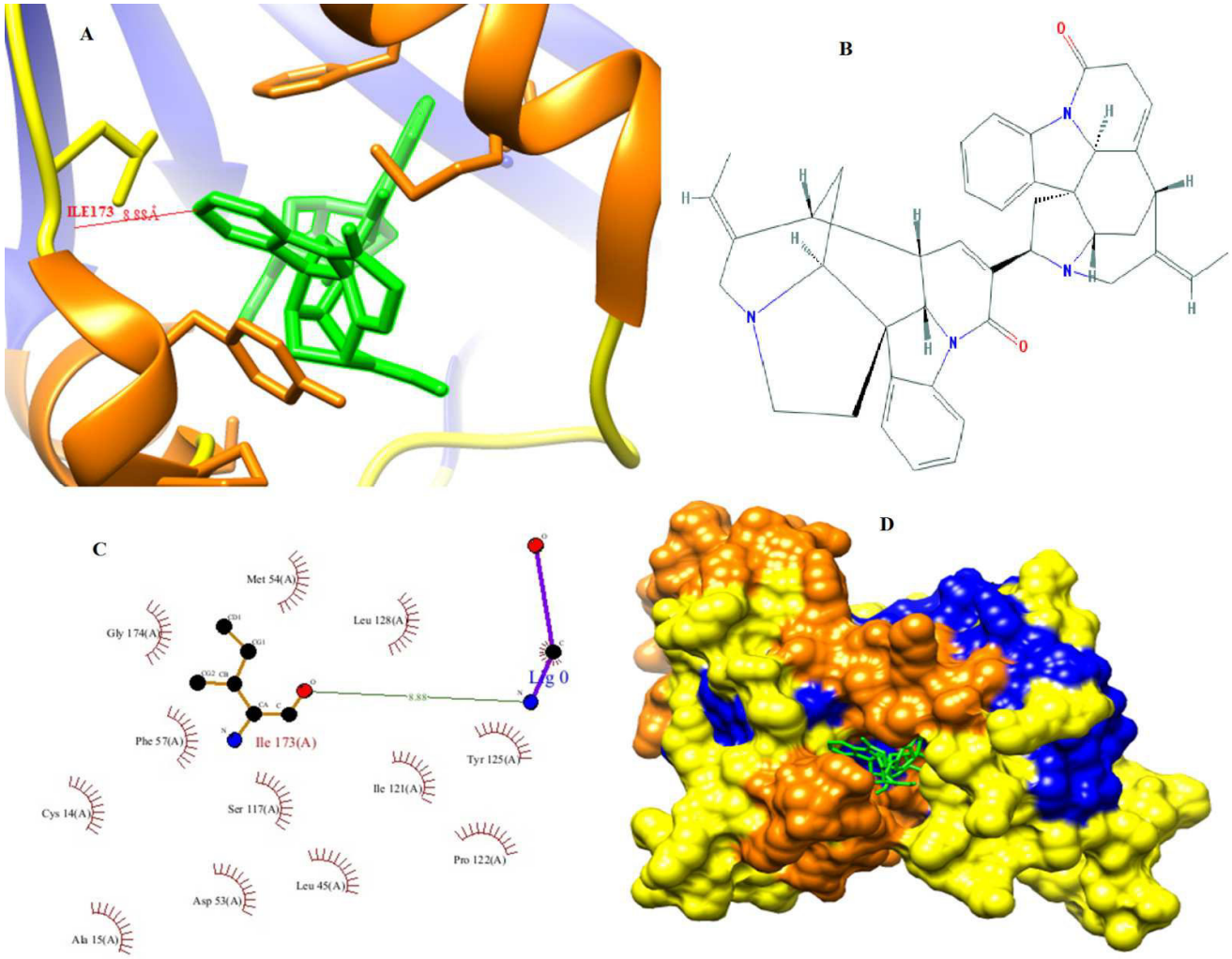

Figure 2
A- Docked Isosungucine on PvDHFR, B- Chemical structure of Isosungucine, C- Ligplot and D- Surface image of PvDHFR cavity with ligand

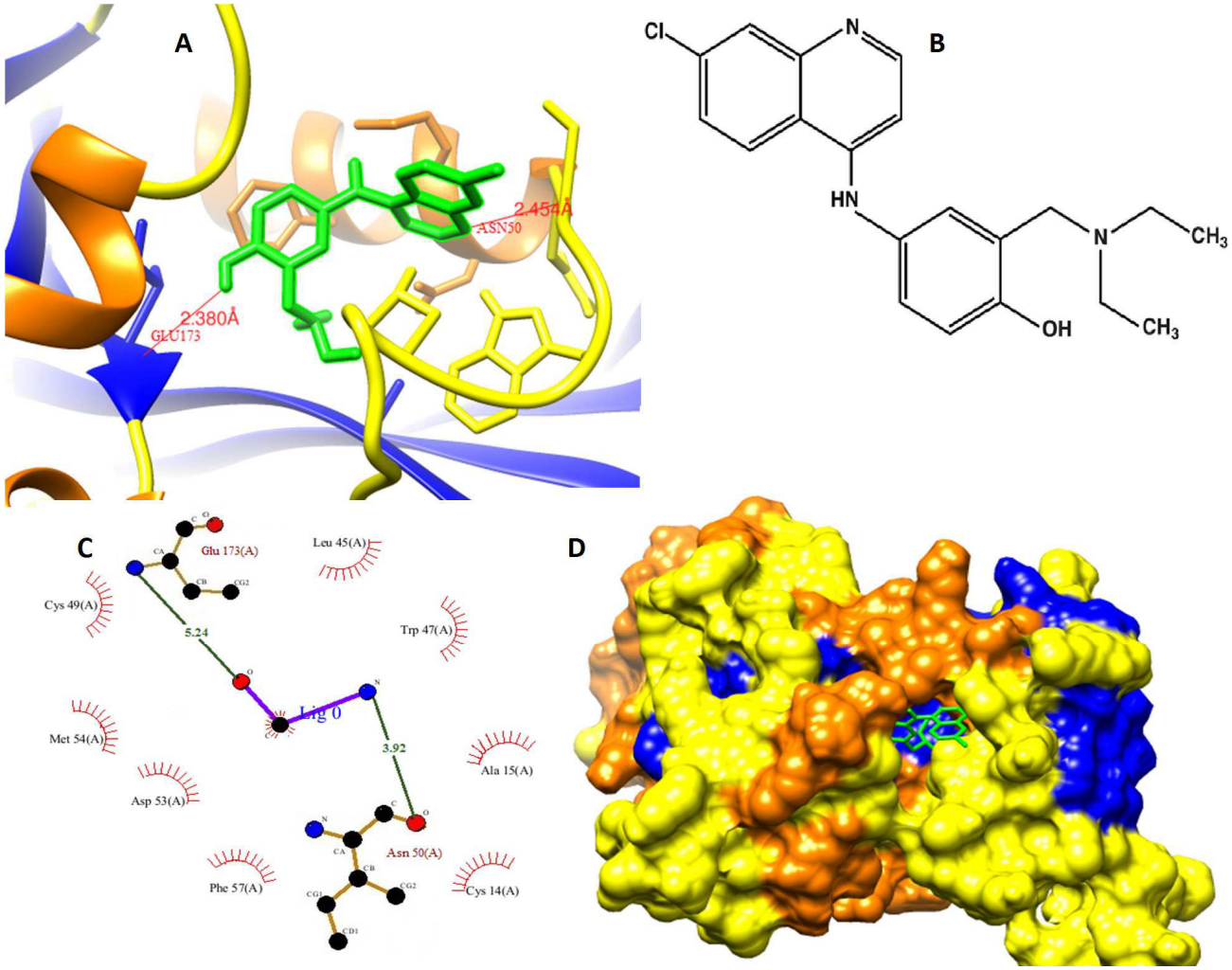

Figure 3
A- Docked Amodiaquine on PvDHFR, B- Chemical structure of Amodiaquine, C- Ligplot and D- Surface image of PvDHFR cavity with ligand

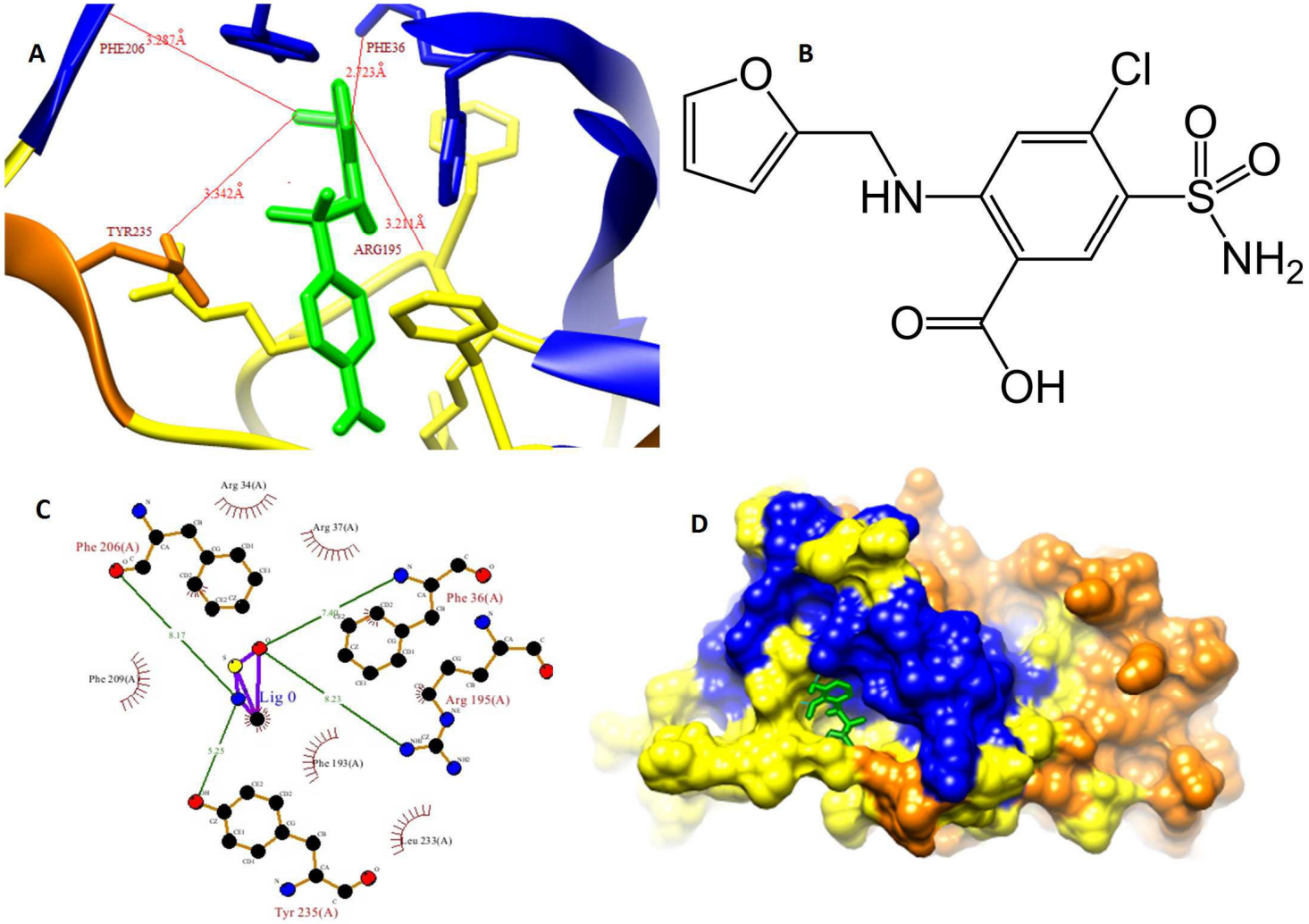

Figure 4
A- Docked Sulfonamide on PvDHFR, B- Chemical structure of Sulfonamide, C- Ligplot and D- Surface image of PvDHFR cavity with ligand

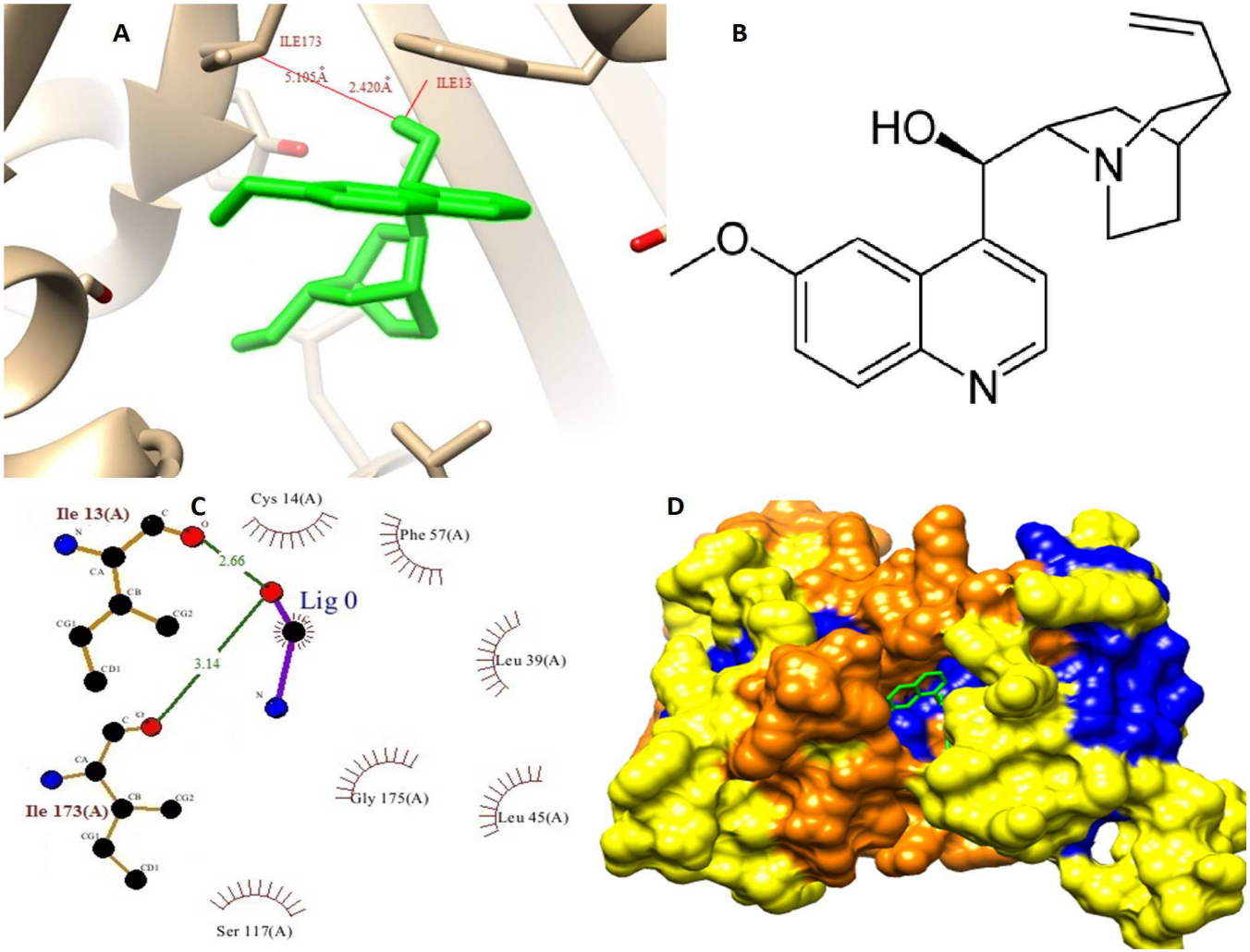

Figure 5
A- Docked Quinine on PvDHFR, B- Chemical structure of Quinine, C- Ligplot and D- Surface image of PvDHFR cavity with ligand

Table 1

Molecular interactions and characteristics of herbal compounds against PvDHFR (pdb id: 2BL9) after docking.

Compound (CID)	LogP Value	Molecular formula	M.W. (g/mol)	Inter molecular Energy (kcal/mol)	Energy Score (kcal/mol)	No. of H-bonds	Hydrophobic contacts	Inhibitor constant K_i at 298.15 K
CID_44576182 (bala)	**0.8**	**$C_{45}H_{72}O_{17}$**	**885.04**	**-23.70**	**-21.31**	**3 (Ser120, Ser58, Arg131)**	**10**	**237.79 aM**
CID_5471853 (isos)	3.5	$C_{42}H_{42}N_4O_2$	634.80	-12.88	-12.88	1 (Ile173)	12	597.16pM
CID_44257959 (cass)	-0.3	$C_{27}H_{28}O_{14}$	576.50	-13.41	-9.83	**6** (Ser58, Arg131, Ser120, Ser117, Tyr179)	10	0.062µM
CID:21575978	1.7	$C_{26}H_{44}O_8$	484.62	-12.63	-9.05	**2** (Asn50, Trp47)	13	0.233 µM
CID:57988022	2.1	$C_{20}H_{18}O_9$	402.35	-10.82	-8.73	**3** (Tyr235, Phe36, Thr35, Arg195)	5	0.397 µM
CID:21597873	2.5	$C_{21}H_{20}N_2O_2$	332.39	-8.60	-8.01	**2** (Ile173, Tyr179)	6	1.35 µM
CID:8549 (quin)	2.9	$C_{20}H_{24}N_2O_2$	324.41	-8.78	-7.2	**2** (Ile13, Ile173)	7	4.51 µM
CID:4993 (pyri)	2.7	$C_{12}H_{13}ClN_4$	248.71	-7.95	-7.05	**1** (Ile173)	8	6.74 µM
CID:2165 (amod)	2.6	$C_{20}H_{22}ClN_3O$	355.86	-8.71	-6.62	**2** (Ser120, Asn50)	8	13.96 µM
CID:17134 sulf	0.7	$C_{12}H_{14}N_4O_4S$	310.32	-8.34	-6.56	**4** (Arg195, Phe36, Tyr235, Phe206)	5	15.67 µM
CID:6178111 (prog)	1.5	$C_{11}H_{16}ClN_5$	253.73	-6.93	-6.34	**2** (Asn50, Asp53)	6	22.71 µM
CID:2719 (chlo)	4.6	$C_{18}H_{26}ClN_3$	319.87	-8.56	-6.17	**1** (Ile173)	8	29.95 µM

bala- Balanitin 6, isos- Isosungucine, cass- Cassiaoccidentalin B, Quin- Quinine, chlo- Chloroquine, amod- Amodiaquine, pyri- Pyrimethamine, prog- Proguanil, sulf- Sulfadoxi

DISCUSSION

1500 herbal compounds and their derivative have been virtually screened and used for molecular interactions with active Site1 residues in PvDHFR, using AutoDock 4.2, which might though to provide inhibitory activity of PvDHFR protein. These interacting molecules are analyzed and compared in close proximity to FDA approved drugs such as amodiaquine, sulfonamide and quinine, which are used to treat malaria. Balanitin-6 have shown highest minimum binding energy of -21.31 Kcal/mol, a LogP value of 0.8, intermolecular energy of -23.70 Kcal/mol and most importantly inhibition constant value (Ki) of 273.79 aM. Ki is an indication of how potent Balanitin 6 inhibitor is and very low concentration might be required to produce half maximum inhibition. Here hydrogen bond (Ser58, Ser120, and Arg131) was found between Balanitin-6 and PvDHFR, into active Site1. The hydrogen bond was formed with nitrogen and oxygen atom present in PvDHFR through Arg131 residue. The inhibition constant identified 10^{12} times higher than the FDA approved drugs such as amodiaquine, chloroquine, proguanil, pyrimethamine, sulphonamide and quinine and the molecular interactions of Balanitin-6 with PvDHFR were found to the same site as for the FDA approved drugs. Almost all selected herbal compounds bound to Sit1 but derivative of cassiaoccidentalin B with CID: 57988022 and derivative of ursonic acid with CID: 9857648 are found in bind to Site2, which is an active pocket for sulfadoxine drugs. Isosungucine bound to Ile173 residue of binding pocket 1 through oxygen and nitrogen residues which were attached with hexagonal ring of the ligand. Isosungscine and its derivative CID: 21597873 both bound with Ile173 which is exactly same binding site, which have been identify for FDA approved drugs like quinine, chloroquine, pyrimethamine. Maximum 5 hydrogen bonds were formed by Cassiaoccidentalin B with Ser58, Arg131, Ser120, Ser47, and Tyr179 residues in PvDHFR. Balanitin-6 derivative CID: 21575978 have shown maximum number of hydrophobic interactions i.e. 13 with Ser117, Gly174, Tyr179, Ile173, Gly175, Ile13, Cys14, Leu39, Leu45, Phe57, Asp53, Ala15 and Met54 in PvDHFR site1 with binding energy of -9.05 Kcal/mol and two hydrogen bond interactions with residues Asn50 and Trp47. Balanitin-6, isosungucine, cassiaoccidentalin-B have higher molecular weight, which seems violating Lipinski's rule of five, but they might be potential antimalarial compounds in line with posaconazole (anti fungal), refamine, amikacin, copreomycin etc which are anti-tuberculosis drugs but do not follow Lipinski's rule of five.

CONFLICT OF INTEREST: Authors do not have any conflicts.

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