DOCKING OF FEW *PLASMODIUM* ENZYMES USING SWISSDOCK

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Abstract: Artemisinin based derivatives are currently in vast use as an antimalarial agents. In spite of this we need more novel antimalarial agents, as development of resistance against artemisinin compounds is already reported in at Thailand – Myanmar boreder and Combodia in *Plasmodia*. In this study we took help of in-silico approach to find out possible antimalarial components present in tulsi and mamejavo plants using Swissdock and ZINC database, as they are traditionally used in malaria treatment. Enzymes namely dihydroxy pteroate synthetase (*dhps*), dihydroxyfolate reductase (*dhfr*) (2BL9) and thymidylate kinase (2YOF) either from *Plasmodium vivax* or *Plasmodium falciparum* were docked against 11 components, as they are key enzymes in metabolism of *Plasmodium*. Among all best results against all 4 proteins were given by laminoribiose, but it violates Lipinski's rule of five. Next to that best results were given by catechin and swertiamarin for 2BL9 and 2YOF. No promising results were found for any *dhps*. In silico drug docking may prove a better approach for any drug designing before going through any in vivo or in vitro analysis. It may really speed up the process of finding new drug agents and even cost cutting, which are necessities of recent time.

Index Terms: *Plasmodium*, *dhps*, *dhfr*, Thymidylate kinase, Swissdock

I. INTRODUCTION:

Malaria is the most important parasitic disease in humans, with transmission occurring in over 100 countries with a population of 3 billion people. The disease causes 1 to 2 million deaths each year equal to 150 to 300 deaths per hour (Breman, 2009; WHO, 2008). World Health Organization forecasts 16% growth in malaria cases annually. These estimates rank malaria as one of the top three killers among infectious diseases (Sachs and Malaney, 2002).

Discovery of CQ was proved revolutionary for treatments of malaria, pushing quinine to the sidelines. But Resistance began from 2 epicenters - Columbia and Thailand in early part of 1960s. *P. vivax* is generally very sensitive to chloroquine-CQ, although resistance is prevalent and increasing in some areas, notably Oceania, (Irian Jaya, Myanmar, Papua New Guinea and Vanuatu), Indonesia and Peru. Since then, resistance has been spreading worldwide and reached the Indian state of Assam in 1973 (Wellems and Plowe, 2001; Wongsrichanalai *et al.*, 2002). Resistance to pyrimethamine has increased rapidly in some areas, and sulfadoxine-pyrimethamine is consequently ineffective Drug-resistant *Plasmodium falciparum* is a particularly serious problem in Southeast Asia, where strains are commonly resistant to CQ, antifolates, quinine, and mefloquine. Resistance is conferred by a stable mutation which is transferred to the progeny (CDC, 2011). Artemisinin and its derivatives (artesunate, artemether, artemotil, dihydroartemisinin) produce rapid clearance of parasitaemia and rapid resolution of symptoms and there by mainly used to treat malaria now a days. But resistance to the artemisinin, was reported along the Thailand - Myanmar border and also confirmed in Cambodia in 2006 (Sinha, 2012).

This explains why development of new antimalarials is constantly required. Primary methods involve use of natural products and synthetic molecules (Krettli, 2009; Krettli *et al.*, 2009). Components of tulsi – *Ocimum sanctum* and mamejavo – *Enicostema littorale* were used in this *in silico* study. Tulsi has many pharmacological activities like hypoglycemic, immunomodulatory, antistress, analgesic, antipyretic, antiinflammatory, antiulcerogenic, antihypertensive, CNS depressant, radio protective, antitumor etc. The active components of herb tulsi chiefly include eugenol, caryophyllene, flavanoids, linalool, elemene, carvacrol etc (Das and Vasudevan, 2006). Mamejavo herb commonly used as a bitter tonic and is also reported to possess antitumor, hypoglycemic and antimalarial activities. It mainly contains components like catechin, proanthocynidin, laminaribiose, ajmalicine, swertiamarin, luteolin etc (Katewa and Arora, 2001).

Dihydroxypteroate synthetase-*dhps* currently also is being used as a target by sulfadoxine-pyrimethamine. Unusual bleeding, rashes, fever, sore throat are major side effects of sulfadoxine-pyrimethamine suggest need for alternate drug. As *dhps* is a proved target to kill malaria parasite we used it in this study. *dhps* enzyme is important in folic acid synthesis pathway and thereby nucleotide synthesis and is important enzyme for parasite. Moreover it is absent in humans. Another enzyme used was dihydroxyfolate reductase-*dhfr* (2BL9). It functions to reduce dihydrofolic acid to tetrahydrofolic acid and thus is also important in folic acid synthesis pathway. Next enzyme was thymidylate kinase (2YOF), which one is a key enzyme in pyrimidine nucleotide biosynthesis (Cui *et al.*, 2012). Thus, altogether we tried to use enzymes directly or indirectly used in nucleotide synthesis. Docking study was performed using online tool Swissdock and component structures derived from ZINC database. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule (Prakash *et al.*, 2010). Swissdock, a web server dedicated to the docking of small molecules on target proteins. It is based on the EADock DSS engine, combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files (Grosdidier *et al.*, 2011).

II. MATERIALS AND METHODS:

Total four protein structures were used in this study and docking was performed using Swissdock against 11 components out of 15. Protein structure files of *Plasmodial* proteins with PDB ID 2BL9 (*P. vivax*) and 2YOF (*P. falciparum*) retrieved from PDB (http://www.rcsb.org). Amino acid sequences of dihydroxypteorate synthetase - *dhps* were retrieved from the data base NCBI in FASTA format (Acession number: AAA19963.1-*P. f*; ABD36501.1-*P. v*) and were modeled for getting 3D protein structure with the help of Swiss model (www.swissmodel.expasy.org/) (http://www.ncbi.nlm.nih.gov/pccompound; http://www.swissmodel.expasy.org/). ZINC database was used for data mining of structures of chemical components of *Ocimum sanctum* and *Enicostema littorale* (http://www.zinc.docking.org/). The 3D protein structures from PDB and Swissmodel were docked with Swissdock with different chemical components by using default parameters. The values were obtained in terms of energy Kcal/mol (http://www.swissdock.ch). Swissdock was automatically able to search the chemical structure from ZINC database. Tulsi and Mamejavo components like eugenol, linalool, catechin, carvacrol, laminaribiose, ajmalicine, swertaimarin, apigenin, luteolin, myricetin, apigenin were retrieved from it. Chemical properties and bioactivity of chemical components were obtained through molinspiration (http://www.molinspiration.com). In online software Swissdock protein structures were browsed in it. On giving chemical name, it automatically searches the structure from Zinc database, the list is displayed and selected molecule is docked at various sites of target protein. The results were obtained at email ID provided. The results were obtained in terms of full fitness/ΔG Kcal/mol. Results were analyzed at the link provided in email or with UCSF Chimera started by a single click, and the predicted binding modes were automatically loaded in its View Dock plug-in.

III. RESULTS AND DISCUSSION:

PDB has a very less number of confirmed three dimensional protein structures. *Plasmodium vivax* or *P. falciparum dhps* is not among them. With Swiss-model, NCBI derived *dhps* FASTA format sequences were modeled as three dimensional structure as shown below in Fig. 1 and 2.

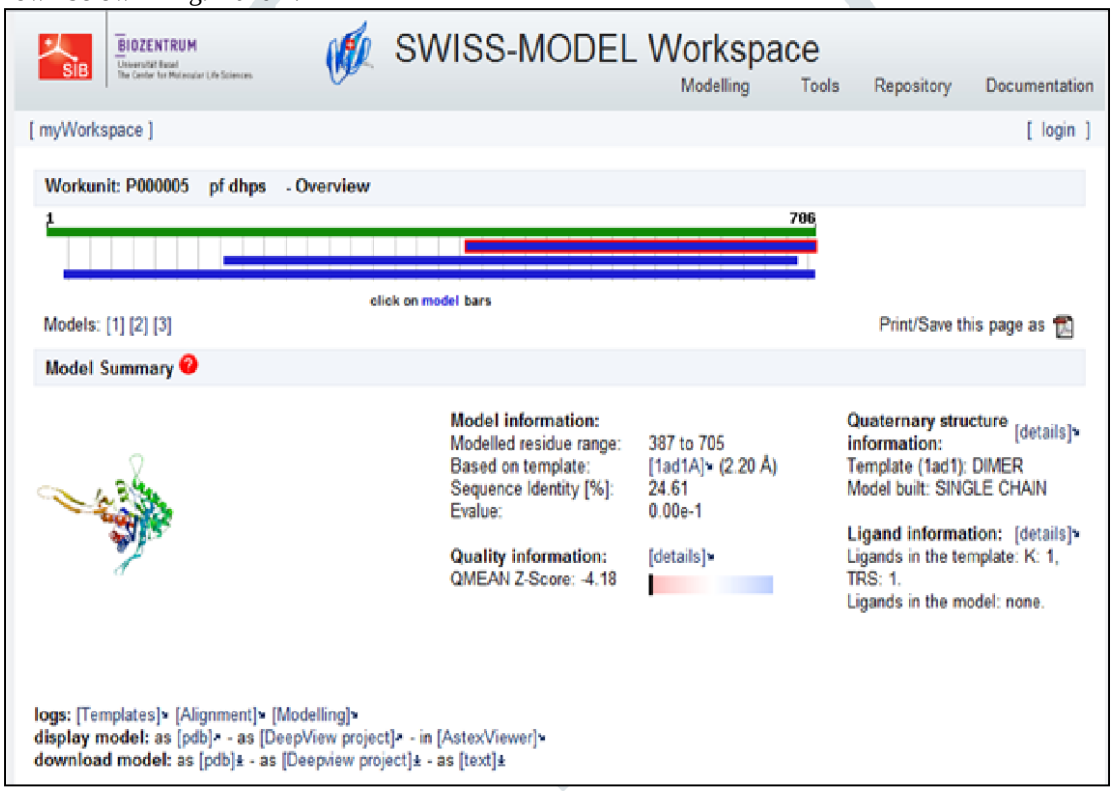

Fig. 1: Outcome of *dhps* Modeling of *P. f* by Swiss-Model

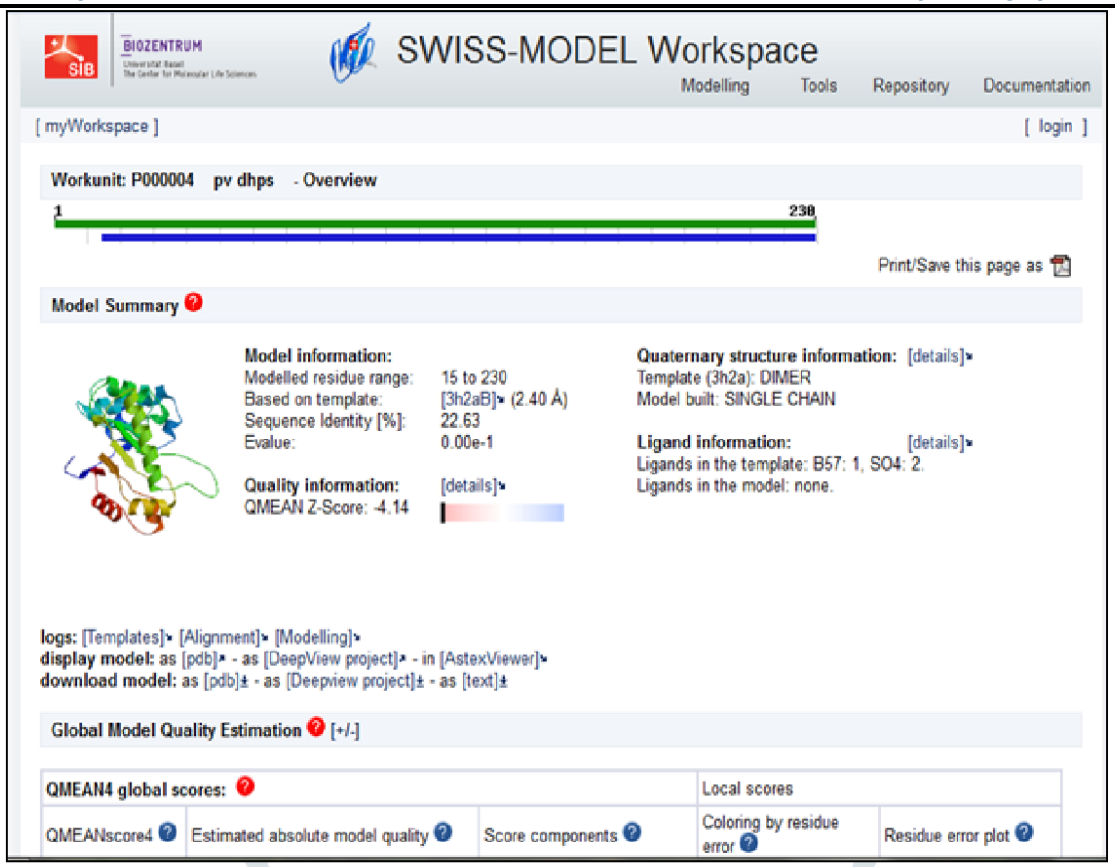

Fig. 2: Outcome of *dhps* Modeling of *P. v* by Swiss-Model

Lipinski stated rule of five for any drug candidate chemical component. Lipinski rule of five helps in distinguishing between drug like and non-drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the rules like High lipophilicity (expressed as Log P less than 5), Molecular mass less than 500 Dalton, Less than 10 hydrogen bond acceptors, Less than 5 hydrogen bond donors etc (Lipinski *et al.*, 2001). Molecules violating more than one of these rules may have problems with bioavailability. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures. Molecular Polar Surface Area-TPSA is calculated as a sum of fragment contributions (Ertl *et al.*, 2000). O- and N- centered polar fragments are considered. TPSA has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration. As shown below in Table 1 Log P was found greater than 5 in oleanolic acid, elemene and caryophyllene. Proanthocynidin A is the only component contains molecular weight greater than 500. Laminaribiose and proanthocynidin A contains more than 10 hydrogen bond acceptor. Myricetin, laminaribiose and proanthocynidin A showing presence of more than 5 hydrogen donor. Laminaribiose and proanthocynidin A also found to have high TPSA. Briefly components from no. 11 to 15 do not follow Lipinski's rule of five.

Table 1: Molecular and Physiological Properties of Chemical Components

Sr. No.	Chemical Component	Log P	Molecular Weight	No. of H Bond Acceptors	No. of H Bond Donors	TPSA
1	Ajmalicine	0.25	353.4	05	02	55.76
2	Apigenin	1.97	286.23	06	04	111.12
3	Apigenin 2	2.20	112.20	00	00	00
4	Carvacrol	3.81	150.22	01	01	20.23
5	Catechin	1.32	290.27	06	05	110.37
6	Eugenol	2.1	164.2	02	01	29.46
7	Linalool	3.21	154.25	01	01	20.22
8	Swertiamarin	-1.65	374.34	10	05	155.14
9	Luteolin	1.94	289.29	07	04	11.12

10	Myricetin	1.392	318.23	08	06	151.57
11	Oleanolic acid	**6.72**	456.71	03	02	57.52
12	Elemene	**5.36**	204.36	00	00	0.00
13	Caryophyllene	**5.17**	204.37	00	00	0.00
14	Laminaribiose	-4.45	342.29	**11**	**08**	**189.52**
15	Proanthocynidin A	3.05	**592.55**	**12**	**09**	**209.75**

(**Note:** Components in **Violet colour** follows the rules; Components in **Red colour** disobey the rules)

Swissdocking was not performed for 4 components namely oleanolic acid, elemene, caryophyllene and proanthocynidin A, as they were not present in ZINC database. Swissdock gave results in terms of full fitness/ΔG Kcal/mol shown in Table 2. In Swissdocking out of 11 components laminaribiose was found most promising against all four enzymes, but was not acceptable as it disobeys Lipinski's rule. Apart from laminaribiose catechin and swertiamarin showed best results against 2BL9 and 2YOF. Luteolin showed best result for 2BL9. No component was found effective against any of the *dhps*. Fig. 3 and 5 shows docking viewed in plug-in with UCSF-Chimera for 2BL9 and *P. v dhps* respectively, whereas Fig. 4 and 6 shows docking viewed in Jmol for 2YOF and *P. f dhps* respectively.

Table 2: Docking Results of Swissdock (in terms of Full fitness/ΔG Kcal/mol)

Sr. No.	**ZINC ID**	**Chemical/ Protein**	**2BL9**	**2YOF**	***Pv dhps***	***Pf dhps***
1.	3978730	Ajmalicine	-1238.50 /-6.17	-3025.47 /-6.64	-1614.19 /-7.24	-2519.64 /-7.63
2.	967563	Carvacrol	-1220.66 /-6.63	-3001.59 /-6.45	-1581.60 /-6.54	-2483.34 /-6.22
3.	1411	Eugenol	-1198.37 -6.73	-2980.06 /-6.69	-1559.78 /-6.45	-2462.59 /-6.37
4.	119983	Catechin	-1213.39 **/-8.23**	-2989.21 **/-8.19**	-1574.01 /-7.57	-2474.56 /-7.06
5.	4095786	Laminaribiose	-1095.91 **/-8.32**	-2868.84 **/-8.15**	-1469.05 **/-8.10**	-2364.09 **/-8.00**
6.	1529819	Linalool	-1225.33 -6.93	-3009.43 /-7.02	-1586.73 /-6.73	-2486.93 /-6.30
7.	18185774	Luteolin	-1220.43 **/-8.13**	-2995.37 /-7.47	-1585.11 /-7.58	-2485.42 /-7.75
8.	3871576	Apigenin	-1223.28 /-7.70	-3001.95 /-7.25	-1586.84 /-7.34	-2487.10 /-7.41
9.	1845895	Apigenin 2	-1216.63 -5.89	-3002.82 /-6.10	-1578.75 /-5.75	-2483.54 /-5.84
10.	4098354	Swertiamarin	-1163.89 **/-8.50**	-2940.83 **/-8.08**	-1532.75 /-7.67	-2430.07 /-7.42
11.	3874317	Myricetin	-1196.03 /-7.78	-2976.87 /-7.66	-1565.46 /-7.82	-2465.59 /-7.72

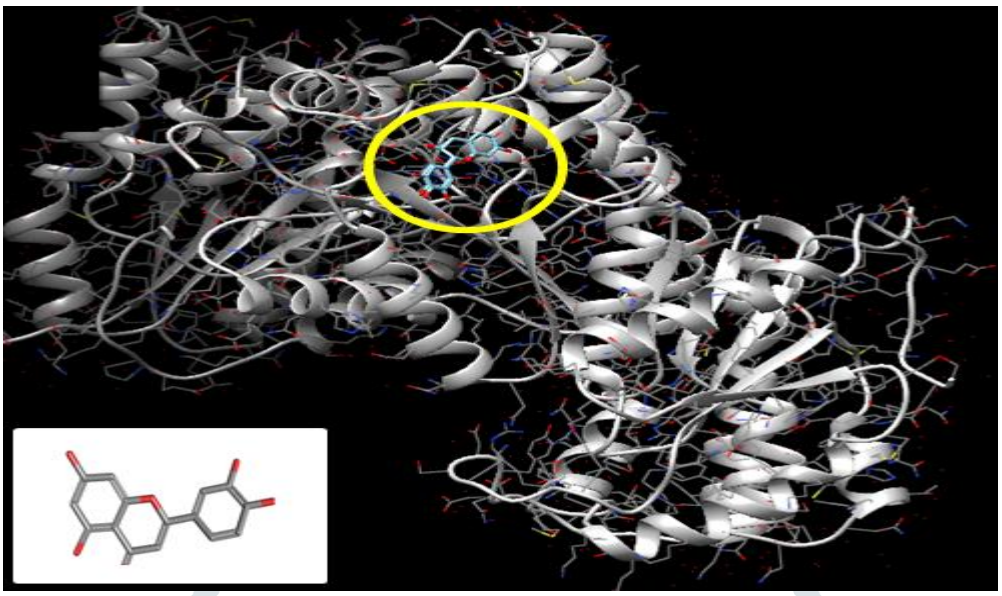

Fig. 3: Docking of 2BL9 with Luteolin (Structure inserted in image) by Swissdock Viewed in UCSF Chimera

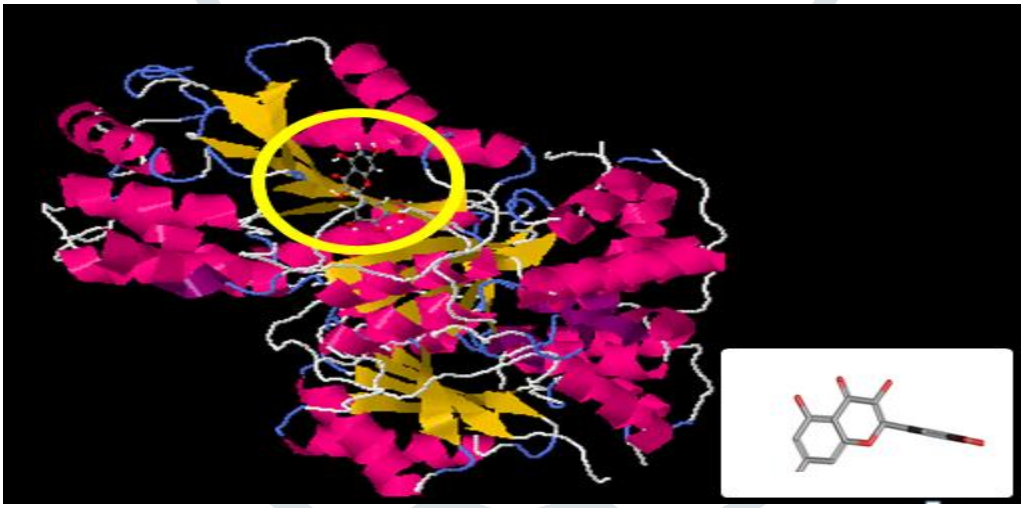

Fig. 4: Docking of 2YOF with Myricetin (Structure inserted in image) by Swissdock Viewed in Jmol

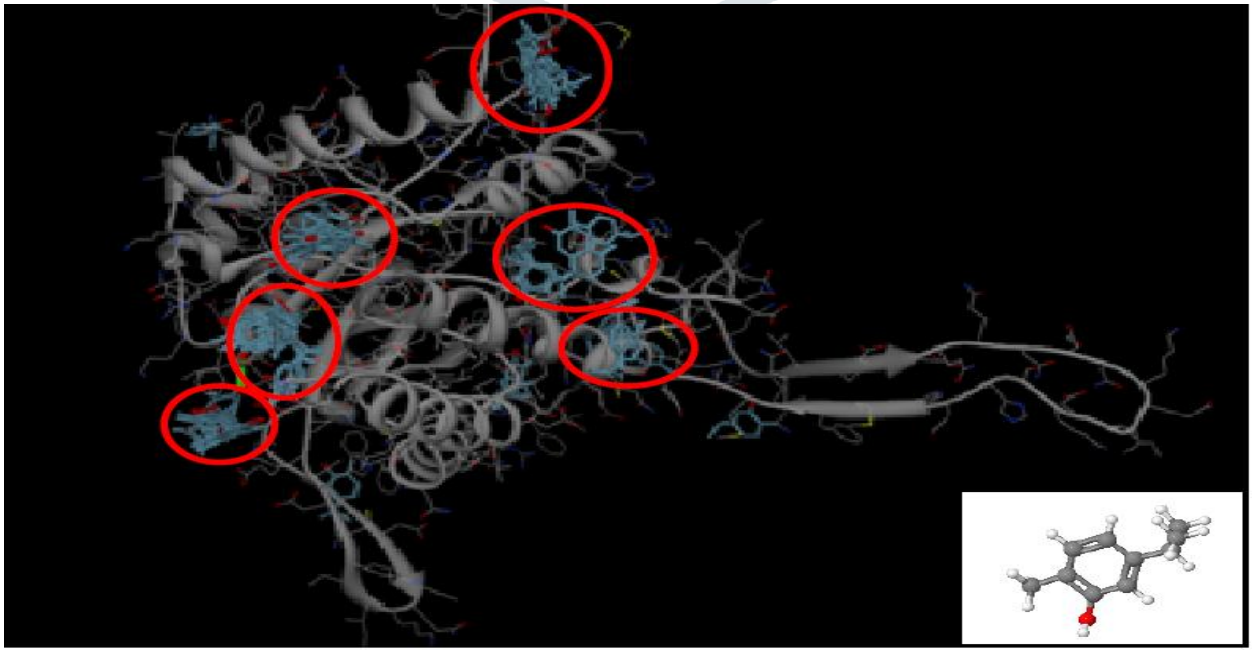

Fig. 5: Docking of *P. v dhps* with Carvacrol (Structure inserted in image) by Swissdock Viewed in UCSF Chimera Showing Various Binding Modes

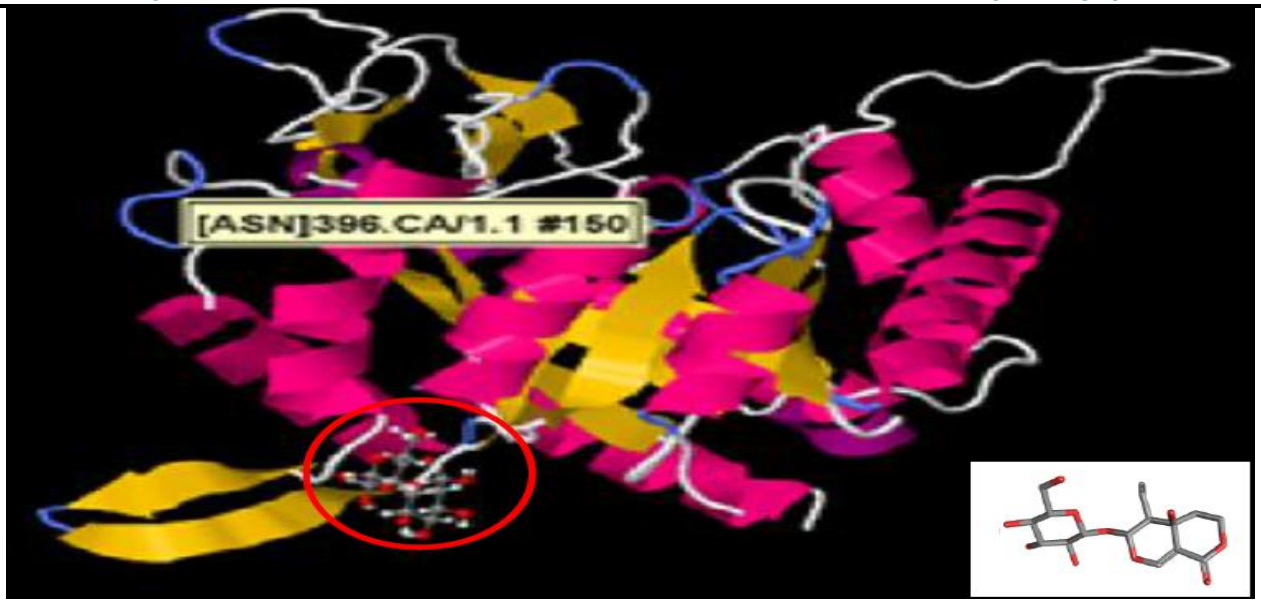

Fig. 6: Docking of *P. f dhps* with Swertiamarin (Structure inserted in image) by Swissdock Viewed in Jmol

In this study we found that both best result giving components catechin and swertiamarin belongs to mamejavo. Even in our previous study performed for Plasmodial LDH, best results were obtained for luteolin, apigenin and swertiamarin, all belongs to mamejavo (Panchal *et al.*, 2013). These chemicals may be originally imparting an antimalarial property of mamejavo in conventional malaria treatment. Docking study on 2 CQ analogues against *p*LDH showed that, these analogues were promising antimalarial components (Aguiar *et al.*, 2012). In one research docking studies were performed to select potential inhibitors of *p*LDH, which were then tested for antimalarial activity against *P. f in vitro* and *P. berghei* malaria in mice. The positive results of these activity trials confirmed that molecular docking studies are an important strategy for discovering new antimalarial drugs (Penna-Coutinho *et al.*, 2011). Some researchers performed docking study using enzyme *dhfr* against mangrove derived components and found that five compounds namely stigmasterol, triterpenoid, tretinoin, pyrethrin and rubrolide-N were effective against *dhfr* (Senthilraja *et al.*, 2012). Computational screening of some antimalarial agents like artemisinin, curcumin and diarylheptanoids against Histone acetyltransferase-HAT and SERCA enzyme was performed and shown that combination of artemisinin and diarylheptanoids can prove to be better combination for antimalarial therapy (Singh and Misra, 2009). Briefly we would like to say that use of computational screening and bioinformatics approach is time saving, faster and comparatively cheaper approach. It must be used for getting cure of any disease before wet lab drug designing and to discover new horizons of array of drugs.

IV. CONCLUSION:

In-silico approach has the potential not only of speeding up the drug discovery process, but can be helpful in cost cutting and may change the way in which drugs are designed. Many chemical components of herbs can be tested *in silico* prior to drug designing, may give new effective drug against one of the top most killer disease like malaria. One can use many vital proteins and enzymes of pathogen as a target with use of docking tools to discover high potential drugs.

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