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Docking Studies of Components of Tulsi and Mamejavo against *Plasmodium* Lactate Dehydrogenase

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Abstract

WHO estimates rank malaria as one of the top three killers among infectious diseases. Though many drugs are available for treatment of malaria, malarial parasite especially Plasmodium falciparum quickly develops resistance under selective drug pressure. Hence we require an array of new drugs for malaria treatment. Lactate dehydrogenase (LDH) is an essential enzyme that catalyses the interconversion of pyruvate and lactate with concomitant conversion of NADH and NAD+. Being an important enzyme of glycolysis for energy production it may prove good target for antimalarial drugs. In our study we retrieved Plasmodium LDH structures from PDB and used them as a drug target protein. Different structures of components of tulsi – Ocimum sanctum and mamejavo – Enicostema littorale were retrieved from PubChem and Zinc databases and docking was performed using Argus lab and Swissdock softwares. In this comparative study we found Argus lab more effective than Swissdock as it can give the results of e-value. Components of tulsi and mamejavo like apigenin, luteolin, carvacrol and rosmarinic acid with Argus lab and ajmalicine, swertiamarin, laminaribiose, catechin with Swissdock found effective against Plasmodium LDH enzyme in our docking study. We found in silico drug docking a better approach to check utility of any chemical as a drug before going through any in vivo or in vitro analysis to shorten out the experiments and cost cutting.

Key Words: Malaria, LDH, Argus lab, Swissdock, Tulsi, Mamejavo.

Introduction

Malaria, a disease caused by *Plasmodium* species, is one of the oldest and largest health challenges affecting 40% of the world's population[1]. It affects 300-500 million people and kills 1.5-2.7 million people annually[2]. WHO forecasts 16% growth in malaria cases annually. These estimates rank malaria as one of the top three killers among infectious diseases[3]. The increasing incidences of malaria in tropical and subtropical countries reflect the development of drug resistant strains of *Plasmodium* and justify referring to malaria as a re-emerging disease[3,4,5]. The antimalarial treatment recommended for *P. falciparum* consists of drug combinations containing artemisinin derivatives (ACT) with other antimalarials, including quinoline compounds, such as amodiaquine and mefloquine. The quinolines act mainly by inhibiting hematin polymerization, thus intoxicating the parasite with the ferriprotoporphyrinic groups generated by hemoglobin degradation. Other antimalarials used in ACT, for example, pyrimethamine and proguanil, inhibit the tetrahydrofolic acid cycle and thus eliminate an important cofactor for DNA synthesis.

Despite the arsenal of drugs available for malaria treatment, the disease remains a worldwide public health problem. *P. falciparum* quickly develops resistance under selective drug pressure[6]. *P. vivax*, the most prevalent human parasite worldwide, has been shown to be resistant to chloroquine[7].

Continuous efforts on the development of new antimalarials are required, and primary methods involve use of different approaches, such as testing natural products and synthetic molecules[8,9]. We selected components of tulsi – *Ocimum sanctum* and mamejavo – *Enicostema littorale* in our *in silico* study. Tulsi herb has been known from as early as Vedic period. It has numerous pharmacological activities like hypoglycemic, immunomodulatory, antistress, analgesic, antipyretic, anti-inflammatory, antiulcerogenic, antihypertensive, CNS depressant, radio protective, antitumor etc. The active components of herb tulsi chiefly include eugenol, caryophyllene, flavanoids, linalool, elemene, carvacrol etc.[10]. Mamejavo herb commonly used as a bitter tonic also called as chhota chirayta (in Guajarati language called kadvu kariyatu). It is also reported to possess antitumor, hypoglycemic and antimalarial activities[11]. It mainly contains components like catechin, proanthocynidin, laminaribiose, ajmalicine, swertiamarin, luteolin etc.

We have used *Plasmodial* LDH in our docking studies as it is an important enzyme of glycolysis and essential for energy production in an *Plasmodia*. Moreover *Plasmodia* lack functional Krebs cycle during some erythrocytic stages[12]. Lactate dehydrogenase (LDH) is an essential enzyme that catalyses the interconversion of pyruvate and lactate with concomitant conversion of NADH and NAD+[13]. The parasite lactate dehydrogenase is a tetrameric enzyme containing 316

amino acids and present in all 4 human parasitic *Plasmodium* species. It has been noted that *p*LDH has notable structural and kinetic properties making it different from mammalian and bacterial LDH[14,15].

We performed comparative docking study using two softwares namely Argus lab and Swissdock and databases PubChem and Zinc for getting structures of chemical constituents. Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules. 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[16]. Argus lab offers quite good on-screen molecule-building facilities, with a moderate library of useful molecules. It is a free molecular modeling package that runs under Windows[17]. 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[18].

Material and Methods

In the first step protein structure files of *Plasmodium* LDH with PDB ID 2AA3 (*Plasmodium vivax*), 1CET (*P. falciparum*), 1T2C (*P. falciparum)* and 1T2F (*Homo sapiens*) retrieved from PDB[19] containing resolution about 2.05, 2.05, 2.01 and 3.00 A° respectively. All 4 sequences were multiply aligned by T-Coffee tool of EMBL database[20]. The target proteins were visualized with RasMol. In next step chemical structures were retrieved from PubChem[21] for Argus lab and Swissdock was automatically able to search the chemical structure from Zinc database[22]. The chemical structures of tulsi and mamejavo retrieved from databases like linalool, catechin, carvacrol, caryophyllene, rosmarinic acid, ajmalicine, luteolin, eugenol, apigenin, swertiamarin etc. were used for docking studies by Argus lab and Swissdock[23,24]. The structures were docked with receptor in Argus lab using default parameters. The values were obtained in terms of energy (e-value) Kcal/mol. Lesser the e-value greater the acceptability of chemical as a drug. In online software Swissdock, protein structures were browsed in it. On giving chemical name, it automatically searches the structure from Zinc database, the list was displayed and selected molecule was docked at various sites of target protein. The results were obtained at email ID provided by us. Results can be analyzed at the link provided in email or UCSF Chimera can be started by a single click, and the predicted binding modes are automatically loaded in its ViewDock plugin[18].

Figure - 1
Multiple sequence alignment by T-Coffee between *Plasmodium* and human LDH sequences

Results and Discussion

Multiple sequence alignment of human and *Plasmodial* LDH by T-Coffee is found in figure 1 showing significant dissimilarity in sequences of them. Docking results of various chemical components of tulsi and mamejavo by Argus lab and Swissdock are listed in table 1 and 2 respectively. With Argus lab we found apigenin, luteolin, carvacrol and rosmarinic acid as effective constituents against target protein LDH with e-values -9.95, -10.2, -9.52 and -8.34 Kcal/mol respectively. Wherever the chemical structures not properly fitted in target protein software shows message, "No acceptable ligand poses were found". Swissdock gave results in terms of full fitness and ΔG Kcal/mol. From Swissdock study best full fitness was found for ajmalicine for all 3 targets (-4161.74, -1159.44, -1296.38). Best ΔG Kcal/mol were found with swertiamarin, laminaribiose, catechin about -8.13, -7.79, -7.87 respectively.

Figure – 2
Structure of *plasmodial* LDH A Chain in RasMol

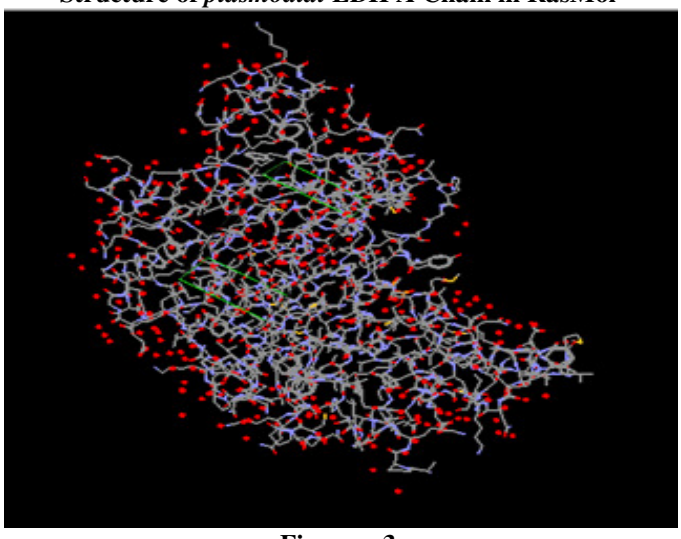

Figure – 3
Docking of 1T2C LDH with Argus lab

Figure - 4
Docking of 1CET LDH with Swissdock viewed in Jmol

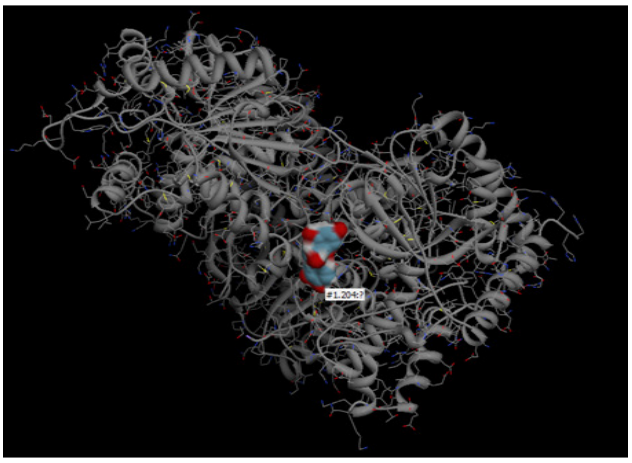

Figure – 5
Docking of 2AA3 LDH with Swissdock viewed in UCSF Chimera

We found Argus lab more reliable as it gives results in terms of e-values and is also not showing results when there is no suitability of chemical with target protein for binding, where as with Swissdock every time results were obtained in terms of full fitness. There was not a single case, when we did not obtain results.

Conclusion

We found Argus lab more in effective in lead molecule prediction in compare to Swissdock. Our study suggest that tulsi and mamejavo components like apigenin, luteolin, carvacrol, rosmarinic acid, ajmalicine and swertiamarin can be used as a lead molecule against *Plasmodial LDH* enzyme for performing *in vitro* and *in vivo* study. Computational biology and bioinformatics have 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* for drug designing, may give new effective drug against one of the top most killer disease like malaria.

Table - 1
Docking results of Argus lab in terms of total no. of poses/energy Kcal/mol

Pubchem ID	Name of compound	2AA3 LDH	1CET LDH	1T2C LDH
3314	Eugenol	105/-7.59	102/-6.117	44/-6.32
6549	Linalool	-	-	-
9064	Catechin	110/-6.99	110/-6.83	-
10364	Carvacrol	101/-7.97	97/**-9.52**	101/-7.02
10494	Oleanolic acid	-	-	-
10583	Elemene	-	-	-
108065	Proanthocyanidin A	-	-	-
439637	Laminaribiose	-	-	-
441975	Ajmalicine	-	-	-
442435	Swertiamarin	-	-	-
5280443	Apigenin	112/**-9.95**	92/-8.67	-
5280445	Luteolin	112/**-10.2**	102/**-9.24**	-
5281515	Caryophyllene	-	-	-
5281672	Myricetin	108/-7.67	-	-
5281792	Rosmarinic acid	-	-	14/**-8.34**
9548705	Germacrene A	-	-	-

Note: [Desh (-) symbol indicates no acceptable ligand poses were found]

Table - 2
Docking results of Swissdock in terms of Full fitness/ΔG Kcal/mol

Chemical/Protein	2AA3	1CET	1T2C
Ajmalicine	**-4161.74**/-6.40	**-1159.44**/-6.81	**-1296.38**/-6.67
Carvacrol	-4127.04/-6.28	-1114.74/-7.33	-1260.58/-6.29
Eugenol	-4106.81/-6.55	-1103.17/-6.33	-1240.96/-6.59
Catechin	-4116.48/-7.94	-1114.74/-7.33	-1252.57/**-7.87**
Laminaribiose	-4005.56/-7.57	-1006.58/**-7.79**	-1140.02/-7.06
Linalool	-4131.82/-6.43	-1127.29/-6.34	-1267.45/-6.80
Luteolin	-4128.02/-7.39	-1124.73/-7.22	-1259.94/-7.16
Apigenin	-4129.38/-7.39	-1127.27/-7.05	-1265.00/-7.42
Apigenin 2	-4124.85/-5.73	-1122.78/-5.73	-1259.83/-5.76
Swertiamarin	**-4076.97/-8.13**	-1073.34/-7.51	-1213.51/-7.43
Myricetin	-4108.36/-7.50	-1103.23/-7.04	-1239.83/-6.99

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