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Original Article

Biotechnology Section

In silico Prediction of Anti-plasmodial Activity of Spices: Targeting Malarial Proteases

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ABSTRACT

Introduction: Malaria, a tropical disease, caused by Plasmodium is curable; still, the burden of malaria infection exists in third world countries. India, particularly among the South-East Asian region, has the maximum mortality rate. The parasites have developed resistance against antimalarial drugs. Alternatively, plants are the most important and ancient source of the drug and spices, in particular, are being used to treat various ailments.

Aim: To identify the effect of common Indian spices in targeting malarial proteases.

Materials and Methods: A bioinformatics approach was used to target malarial proteases which exhibit an important role in the erythrocytic cycle of the pathogen by degrading Haemoglobin. Proteases, in particular, Falcipain and Plasmepsin were used to perform docking studies to identify potent molecule for inhibition of malarial progression. Data were collected in the form of structural files from PDB (for protein) and PubChem (for ligands). The Protein structures and ligands were prepared and molecular docking had been done to predict interactions.

Results: All the eighteen compounds used in study have shown quite a good affinity with target proteins in the terms of energy (negative binding energy). However, comparatively, interactions of falcipain 2 with thymol and gingerol were almost three times lower than other ligand. Except these, energy ranges were in between 30-40 kilo Joule in negative. Strongest interactions were found in between Plasmepsin 4-piperamide and plasmepsin 2-gingerol. Also, it was found that gingerol was most potent bioactive molecule interacting with almost all proteins as predicted by docking studies.

Conclusion: Plasmepsin 2 and Plasmepsin 4 with gingerol and piperamide with highest cdocker energy might indicate the potential of molecules in targeting these proteases. Also, Gingerol was found to be most potent in interacting with malarial proteases.

Keywords: Bioinformatics, Docking, Falcipain, Herbal

INTRODUCTION

Plants are one of the most important and diverse sources of compounds. Indian culinary spices are known to have various pharmacological activities and are treating ailments from time immortal. The World Health Organisation (WHO) estimated that approximately 65% of the world population relies on plant-derived traditional medicines for the treatment of their primary ailments. Plants are the basis of traditional medicine systems from around 2600 BC in Mesopotamia [1]. Given the paradigm shift of treatment efficacy from traditional biology to medicine, there is a need to utilise the role played by the compounds generated from the spices in various diseases [2]. In addition, molecular modelling, combinatorial chemistry, and other synthetic chemistry techniques are being used by pharmaceutical companies but particularly medicinal plants are still a huge source of new drugs, new drug leads, and New Chemical Entities (NCEs). In beginning years of the 21st decade, approximately one-quarter of the best-selling drugs worldwide were derived from natural products [2].

The vector of malarial parasite *Plasmodium falciparum* is the female Anopheles mosquito. The malaria parasite has a complex life cycle, leading to the challenge of developing a successful medication against it. Another problem is malarial drug resistance against existing targets [3].

Understanding the interplay of host-parasite interaction may provide the basis for the development of a successful drug against it. The malarial life cycle consists of two cycles, an asexual cycle in the human host and a sexual cycle in the mosquito host [4].

The drug discovery for malaria was in large extent been serendipitous. Available antimalarial targets are mainly blocking blood-stage of parasites. The limitations of antimalarial drugs demand the need for new drugs, which should be ideally directed against new targets as age-old targets like are still being used [5].

Among the potential targets for antimalarial therapy are Plasmodium proteases that are drug-able targets, are being used for treating various disorders. Proteases are known to be required for the rupture and subsequent re-invasion of erythrocytes and for the degradation of haemoglobin [6,7]. Because of the high degree of similarity, the specific role of the food vacuole plasmepsins in vivo has been unclear [8-10]. This study was focused to discover a lead molecule from spices which can be used to target malarial proteases. Falcipain and plasmepsins were being used in this study. Molecular docking had been used to predict the interactions between proteins and bioactive molecules. The effect of some spices was established to have anti-malarial activity. Janfaza S and Janfaza E, illustrated that the antimalarial activity of Thymoquinone from oil of *Nigella sativa* has antimalarial properties [11] and also thymoquinone and artemisinin hybrid molecules had shown to have increased potency as tested in vitro [12]. Antimalarial activity of herbal extract and formulations from nutmeg had been proved in vitro [13]. Diosgenone (a compound derived from diosgenin) derivatives reduced Malarial parasitemia in mice by oral administration [14]. Chakrabarti R et al., illustrated inhibitory effect of curcumin [15]. Reddy R et al., showed that curcumin inhibits chloroquine-resistant *Plasmodium falciparum* growth in culture and oral administration in infected mice reduces blood parasitemia by 80-90 percent [16]. Curcumin artemisinin combination therapy can also be used for treating malaria as suggested in vitro [17]. Cinnamic acid derivatives were also reported to inhibit in vitro growth of *Plasmodium falciparum* [18]. Allicin could partially protect host against malaria parasite [19] and also shown to inhibit circumsporozoite protein of Plasmodium [20].

In a country like India, where using plant parts as medicine being a tradition and still most of the population relies on traditional medicine, use of spice derived lead compounds can pave a way for better treatment of malaria. So the present work was aimed to predict the potential lead from spices namely {(Galbanic Acid (*Asafetida*), Eugenol (Basil and clove), Allicin (garlic), Curcumin (Turmeric), Thymol (Carom seeds), Thymoquinone (Black Cumin), Anethole (*Fennel*), Diosgenin (*Fenugreek*), Macelignan (Nutmeg), Piperamide (Black pepper), Pipercide (Black pepper), Capsaicin (Chillies), Gingerol (Ginger), Cinnamic acid (Cinnamon), Cinnamaldehyde (Cinnamon)} that can target malarial Proteases and can provide a better treatment for malaria.

MATERIALS AND METHODS

The current study was completely an *In silico* analysis. It was conducted in the year 2017. No ethical approval was involved as complete study is based on bioinformatics. The study was conducted between February to October 2017 at Birla Institute of Scientific Research, Jaipur, Rajasthan, India as Doctoral research from KIIT University, Bhubaneswar, Odisha, India.

Retrieval of Structural Files of Bio-active Compounds of Spices

The bio-active compounds of spices were known to have medicinal properties [21-25]. These compounds were archived in their three-dimensional structure. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to retrieve structural files in SDF format of these compounds [26]. The study was aimed to find out the effect of common culinary spices used in India against malarial proteases.

Retrieval of Protein Structural Data

RCSB Protein Data Bank (PDB) is one of the major sources of Protein structural data [27]. Structure of Malarial proteins has been retrieved from PDB database. The details of structures taken had been described in [Table/Fig-1] [28-32].

Sr. No.	Protein name	PDB ID	Resolution	Active site origin	Active site	Reference
1.	Falcipain 3	3BWK	2.42 A°	PDB	-6.66929, -37.5781, 49.00 R=10.7	[28]
2.	Falcipain 2	3PNR	2.60 A°	PDB	14.9119, 12.5747, 10.3243 R=17.1	[29]
3.	Plasmepsin 1	3QRV	2.40 A°	Receptor cavity	14.4615, 18.618, 24.092 R=24.3	[30]
4.	Plasmepsin 2	1XDH	1.70 A°	PDB	16.3857, 62.7968, 24.2156 R=11.5	[31]
5.	Plasmepsin 4	1LS5	2.80 A°	PDB	-26.4472, 36.8228, 43.1958 R=11.7	[32]

[Table/Fig-1]: Proteins used for docking Studies [28-32].

Preparation of Protein Structure

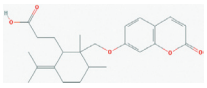
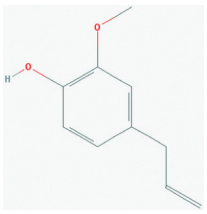
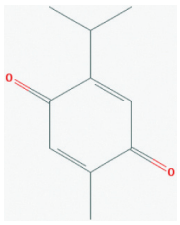
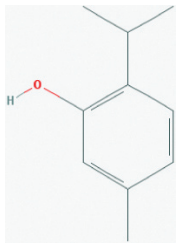
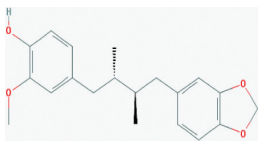
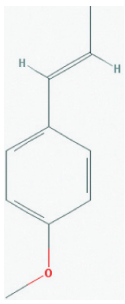
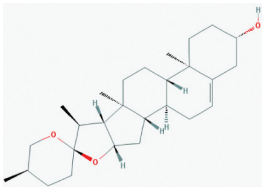
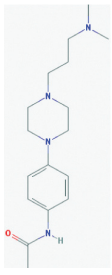
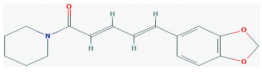
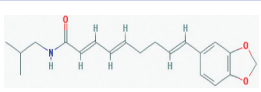
Discovery studio (Version) of Biovia systems (Version 2017 R2) was used for preparing Protein structure. The licensed modules were used for performing all the analysis [33].

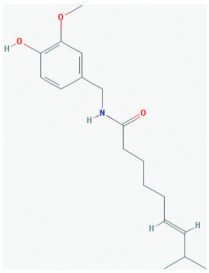
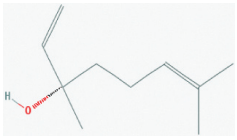
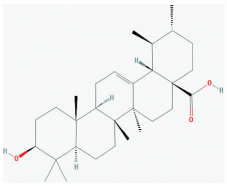
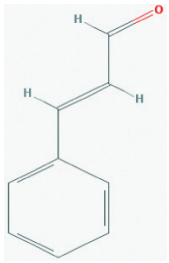
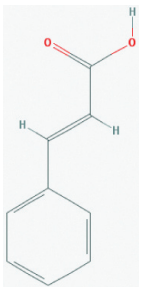
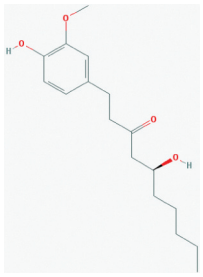
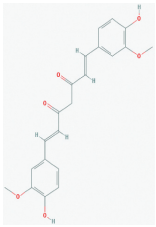
Active Site Prediction

The prediction of active site was done using Discovery studio. Selection was made using active sites present in PDB record. However, Active sites can also be selected using Protein cavities [Table/Fig-2].

Ligand Preparation for Docking

The ligands were prepared using Discovery Studio produced stereoisomers; tautomer checked charges and ligand/ligands were ready for docking. Already known bio-active molecules from different spices were retrieved from the PubChem Database in SDF format and merged into same Spatial Data File (SDF) file. The molecules were then prepared using Ligand preparation tool of Discovery studio.

Active compound (Source spice)	Pub chem ID	Molecular formula and weight (g/moL)	Molecular structure
Galbanic acid (Asafoetida)	7082474	C24H30O5 (398.499)	
Eugenol (Clove/ Basil)	3314	C10H12O2 (164.204)	
Thymoquinone (Black cumin)	10281	C10H12O2 (164.204)	
Thymol (Carom seeds)	6989	C10H14O (150.221)	
Macelignan (Nutmeg)	10404245	C20H24O4 (328.408)	
Trans-Anethole (Fennel)	637563	C10H12O (148.205)	
Diosgenin (Fenugreek)	99474	C27H42O3 (414.63)	
Piperamide (Black pepper)	14739	C17H28N4O (304.438)	
Piperine (Black pepper)	638024	C17H19NO3 (285.343)	
Retrofractamide B (Pipercide) (Black pepper)	5372162	C22H29NO3 (355.478)	

Capsaicin (Chillies)	1548943	C18H27NO3 (305.418)	
Coriandrol (Coriander)	67179	C10H18O (154.253)	
Ursolic acid (Basil)	64945	C30H48O3 (456.711)	
Cinnamaldehyde (Cinnamon)	637511	C9H8O (132.162)	
Cinnamic acid (Cinnamon)	444539	C9H8O2 (148.161)	
Gingerol (Ginger)	3473	C17H26O4 (294.391)	
Allicin (Garlic)	65036	C6H10OS2 (162.265)	
Curcumin (Turmeric)	969516	C21H20O6 (368.385)	

[Table/Fig-2]: List of Bio-actives and corresponding spices.

Molecular Docking Studies

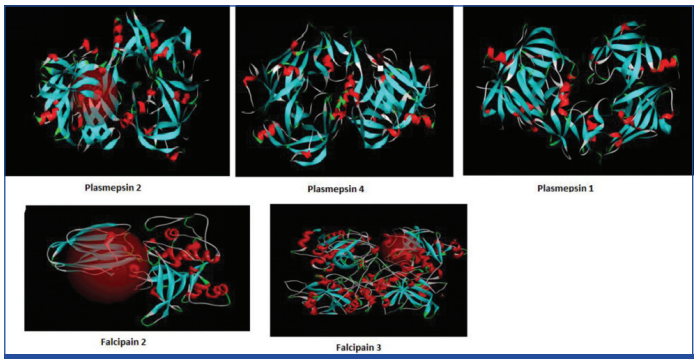
Molecular docking was used to screen compounds from the library of prepared ligands which were showing a reasonable interaction with proteins of interest. Docker method was used for docking. Docker energy was one of the important factors in screening of ligand poses besides the interaction type and the bond length.

RESULTS

Six Structures were Prepared *in silico*

The PDB structures of six proteins, three falcipains and three plasmepsins were analysed using Biovia discovery studio tool prepare protein.

The Prepare Protein protocol prepared proteins for input into other protocols, performing tasks such as inserting missing atoms in incomplete residues, modelling missing loop regions, deleting alternate conformations (disorder), removing waters, standardising atom names and protonating titratable residues using predicted pKs [Table/Fig-3].



[Table/Fig-3]: Structures of plasmepsins and falcipains.

Ligand Analysis and Preparation

A total of 18 compounds were taken for docking studies and ligand preparation tool is used to prepare 18 ligands for docking. The Prepare ligands protocol helped to prepare ligands for input into other protocols, performing tasks such as removing duplicates, enumerating isomers and tautomers, and generating 3D conformations. A total of 22 ligands were generated including tautomers and stereoisomers [Table/Fig-4]. Allicin (PID 65036) had 2 stereoisomers, Curcumin (969516) had 4 tautomers, piperamide had two ionizations and two tautomers for each. A ligand was failed due to duplicate result and that was one of the structures of 969516. So, we had a total of 22 structures to be docked against 5 proteins [Table/Fig-4].

DISCUSSION

Current study was based on semi flexible docking scheme in which protein remains rigid and ligand tries to fit into the active pocket. All the five proteins had been screened against 22 prepared ligands which yielded hundreds of ligand poses [Table/Fig-5,6]. Initially, the ligand with highest Cdocker energy for all proteins was selected. Hydrogen bonds and hydrophobic interaction play an important role in protein ligand interaction. Presence of H bond indicates a strong interaction between protein and ligand [34-36]. The ligand poses with higher Cdocker energy aligns well with the structure. Lowest energy (Highest negative) indicates a more favourable binding of ligand [37-39].

These two criteria were considered here for selecting poses. Hence, interactions of plasmepsin 4-piperamide [Table/Fig-7] and plasmepsin 2-gingerol [Table/Fig-8] simultaneously had highest Cdocker energy. The polar amino acids i.e., Serine and aspartate present at active site of plasmepsins seem to have important role in interaction.

However, if we assume the scenario in context of ligands, only few ligands had shown interaction with malarial proteases, most potent being gingerol, interacting with most of the structure of malarial proteases.

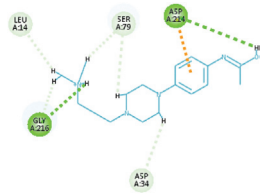
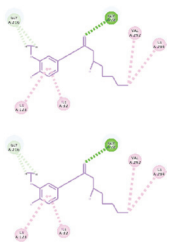
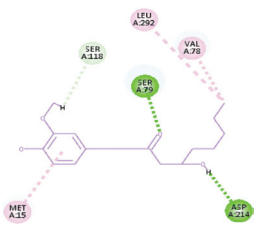
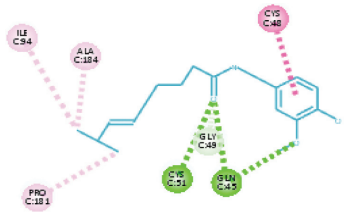
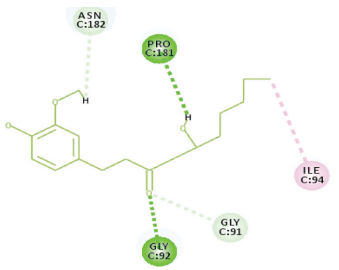
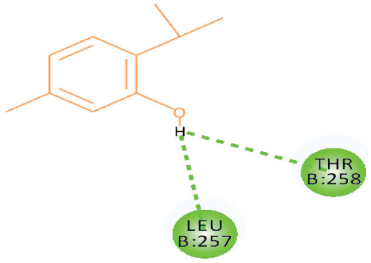
Index	Name	IonizationIndex	NumberOfIonizations	NumberOfStereoisomers	NumberOfTautomers	PUBCHEM_COMPOUND_CID
1	65036	1	1	2	1	65,036
2	65036	1	1	2	1	65,036
3	1548943	1	1	1	1	1,548,943
4	637511	1	1	1	1	637,511
5	5355899	1	1	1	1	5,355,899
6	67179	1	1	1	1	67,179
7	969516	1	1	1	4	969,516
8	969516	1	1	1	4	969,516
9	969516	1	1	1	4	969,516
10	99474	1	1	1	1	99,474
11	7082474	1	1	1	1	7,082,474
12	3473	1	1	1	1	3,473
13	10404245	1	1	1	1	10,404,245
14	14739	1	2	1	2	14,739
15	14739	1	2	1	2	14,739
16	14739	2	2	1	2	14,739
17	14739	2	2	1	2	14,739
18	5372162	1	1	1	1	5,372,162
19	638024	1	1	1	1	638,024
20	3314	1	1	1	1	3,314
21	6989	1	1	1	1	6,989
22	10281	1	1	1	1	10,281

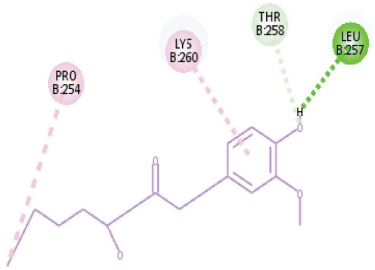
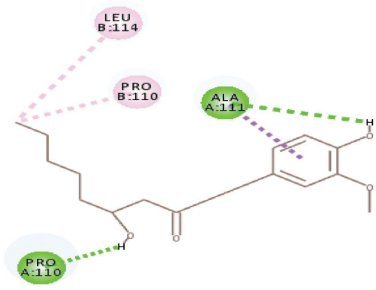
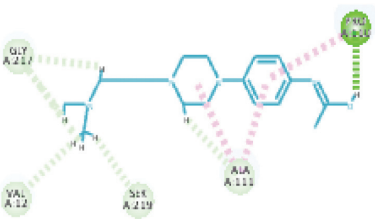
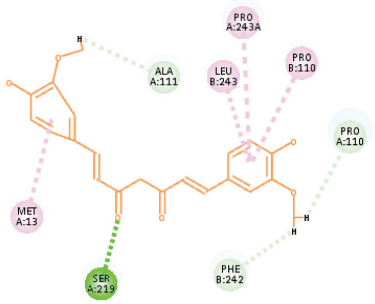
[Table/Fig-4]: Ligands used for docking.

Category	Sub-Category	Type	Color
Hydrogen Bonds	Classical	Conventional Hydrogen Bond	
Hydrogen Bonds	Non Classical	Carbon Hydrogen Bond	
Hydrogen Bonds	Non Classical	Pi Donor Hydrogen Bond	
Hydrogen Bonds	Water	Water Mediated Hydrogen Bond	
Hydrogen Bonds	Water	Water Hydrogen Bond	
Hydrogen Bonds	Salt Bridge	Salt Bridge	
Electrostatic	Charge	Attractive Charges	
Electrostatic	Charge	Salt Bridge	
Electrostatic	Pi-Charge	Pi-Cation	
Electrostatic	Pi-Charge	Pi-Anion	

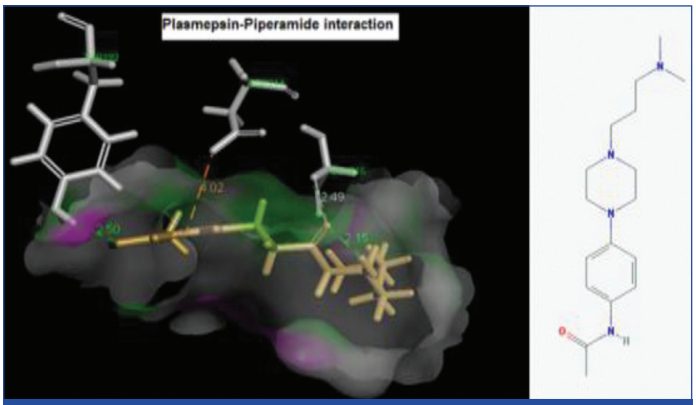
Category	Sub-Category	Type	Color
Hydrophobic	Pi-Hydrophobic	Pi-Pi Stacked	
Hydrophobic	Pi-Hydrophobic	Pi-Pi T-Shaped	
Hydrophobic	Pi-Hydrophobic	Amide-Pi Stacked	
Hydrophobic	Alkyl Hydrophobic	Alkyl	
Hydrophobic	Mixed Pi/Alkyl Hydrophobic	Pi-Sigma	
Hydrophobic	Mixed Pi/Alkyl Hydrophobic	Pi-Alkyl	

[Table/Fig-5]: Color coding scheme for ligand interaction.

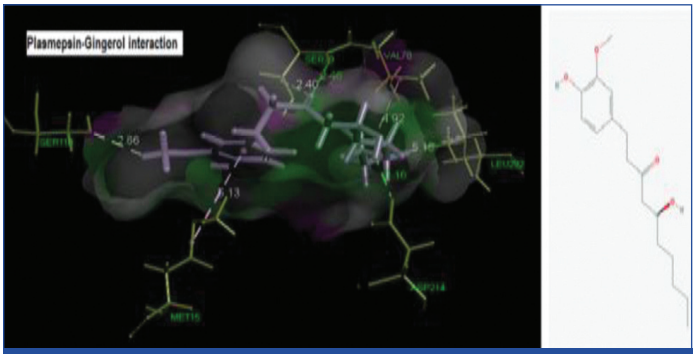
Protein (C Docker energy)	Interacting molecule	2D Interaction map
Plasmepsin 4 (40.8051)	Piperamide	
Plasmepsin 4 (39.5709)	Gingerol	
Plasmepsin 2 (40.4649)	Gingerol	
Falcipain 3 (27.7347)	Capsaicin	
Falcipain 3 (32.3169)	Gingerol	
Falcipain 2 (10.9035)	Thymol	

Falcipain 2 (14.2815)	Gingerol	
Plasmepsin I (32.096)	Gingerol	
Plasmepsin I (31.6361)	Piperamide	
Plasmepsin I (30.5079)	Curcumin	

[Table/Fig-6]: A 2D Interaction diagram of top energy poses.



[Table/Fig-7]: Plasmepsin-Piperamide interaction and Plasmepsin structure.



[Table/Fig-8]: Plasmepsin-Gingerol interaction and Gingerol structure.

CONCLUSION

The docking based virtual screening was done for 22 prepared ligands and for five protease structure (two falcipain and three plasmepsins). Plasmepsin 2 and Plasmepsin 4 with gingerol and piperamide with highest cdocker energy might indicate the potential of molecules in targeting these proteases. Also, gingerol was found to be most potent in interacting with malarial proteases further modification and development of analogues for this ligand may lead to development of better antimalarial drug.

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