

***In Silico* Analysis of Hepatoprotective Properties of Bael Leaves**
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ABSTRACT

Ancient India is one of the pioneers of studies of plants as medicine, i.e. Ayurveda. In our social and economic life we hardly take care of our food we are taking. One such unique herb is Bael which has often been regarded as a brain booster. The whole plant including the flowers can be used for medicinal purposes. It has a bitter and sweet taste and is known to impart a cooling energy. Bael is full of antioxidants that are essential for living a healthy life. We are using this property of Bael to get some new drugs for Tobacco mosaic virus. The uses of various pesticides, preservatives, etc. turn the foods into poison. Moreover the side effects of these pesticides and preservatives, etc. are dangerous as because it leads to initiation of different cancer. In this whole world, the number of patients dying from cancer is increasing in a very threatening way. *In-silico* analysis has done using software and we further targeted some of the genes responsible for Tobacco mosaic virus and pharmacophores from Bael and did some in silico analysis. In this we have found that these two pharmacophores are having better Mol Doc score from any others.

KEY WORDS : ADMET ANALYSIS, BAEL, DOCKING, HEPATITIS

Introduction

Plants have been used for medicinal purposes long before prehistoric period. Indian vedas and European cultures were using medicinal herbs for over 4000 years as medicine. The uses of medicinal herbs has increased during the past three decades and the screening of plant extracts have been done frequently for discovering new drugs(Rajamanickam et al., 2007). Aeglemarmelos(Bael) is a deciduous shrub or small to medium-sized tree belonging to the family of Rutaceae. It is found in India, Pakistan, Bangladesh and Myanmar. The medicinal properties of this plant has been described in Ayurveda. The roots, leaves, bark, seeds and fruits consists medicinal values. The bael leaves are used to treat diarrhea , weakness of heart, blood sugar, backache,ulcers(Dutta et al., 2014). Various compounds such as Skimmianine, Aegelin, Lupeol, Cineol, Citral, Citronellal, Cuminaldehyde, Eugenol, Marmesenin have been refined from leaves of bael which shows anticancer,antiulcer,hepatoprotective,antibacterial,cardioactive ,anti-inflammatory, antiseptic, antimalarial, antiallergic, antihyperglycemic, and antioxidant properties (Maity et al., 2009).

Hepatitis A (HAV),also called hep A, is an epidemic liver infection caused by hepatitis A virus. It is apicornavirus and can commonly spread through consumption after fecal contamination of mucous membranes , skin, food and water(Jennifer A. Cuthbert 2001). Some commonly observed symptoms of HAV are Jaundice (yellow eyes and skin),belly pain, dark urine, loss of appetite , vomiting, pale-colored poop. Hepatitis B(HBV) is a viral infection that can cause both acute and chronic diseases of liver such as liver cancer. It is an epidemic liver infection caused by hepatitis B virus. There are approximately 350 million people worldwide remain chronically infected carriers of this disease among whom 500,000 people die due to liver cancer and cirrhosis (Chien-Jen Chen et al., 2009). Hepatitis B virus belongs to Hepadnaviridae family and commonly transmitted through contact with blood and other body fluids, as well as from mother to child during birth and delivery (world health organization, www.who.int/news-room/fact-sheets/detail/hepatitis-b). The most common symptoms of this disease are Jaundice(skin or the whites of the eyes turn yellow, and your pee turns brown or orange), loss of appetite, nausea, persistent fatigue for months.

Hepatitis C (HCV) is a liver infection caused by hepatitis C virus. It is generally symptomless but can cause appreciable liver damage. About 170 million people worldwide have the disease and is a major health burden as 60-80% of infected people progress to chronic liver infection (AA Modi and TJ Liang , 2008). Some commonly observed symptoms of HCV are dark urine, fatigue, yellow tint in skin or eyes, nausea, fever and it is transmitted through the infected person's body fluid and blood.

The leaf extract of bael contains the chemical compound Eugenol and marmesinin which exhibits pharmacological effects such as antioxidant, antibacterial, hepatoprotective and antiulcer(Maity et al., 2009). Considering the various medicinal properties of bael leaves, the present study was taken on to evaluate the hepatoprotective properties of bael leaves in liver infection such as HAV, HBV and HCV.

Materials and methods

Various pharmacophores of bael leaves have been listed and their respective SDF were taken accordingly from Pubchem, Molinstincts, and Chebi. The enzyme corresponding to microbe of HAV, HBV and HCV have been taken from BRENDA (Braunschweig Enzyme Database). Then, the PDB (Protein Data Bank) code was found from RCSB (Research Collaboratory for Structural Bioinformatics). The above mentioned information was then processed in Discovery Studio to initiate Docking. The following screenshots are taken from Discovery Studio, showing positive results of docking;

Table 1: The list of pharmacophores and the targeted genes from HepatitisGene

Sl.No	Pharmacophores from Bael	Targeted Genes from HepatitisGene	PDB No of the Genes
1	aegeline	Core Protein	5T2P
2	skimmianine(1)	BETA 2-MICROGLOBULIN	1HHH
3	d-limonene	NS3 protease,NS4A protein	3SU4
4	marmelosin	19-mer peptide from Genome polyprotein	3KF2
5	allocryptopine	RNA-dependent RNA polymerase	2XWH

Protein identification and preparation

The reported molecular targets responsible for HepatitisGene are taken (Table 1) and the X-ray crystallographic structures of these target proteins were retrieved from protein data bank (PDB). The retrieved PDB structures contain water molecules, heavy atoms, cofactors, metal ions etc. and these structures do not have information about topologies, bond orders and formal atomic charges. Hence the downloaded PDB structures were prepared using ‘prepare protein’ protocol of Discovery Studio 4.0. The target proteins were prepared by removing all water molecules, ligands and other hetero atoms from the structures. Hydrogen atoms were added to the atoms to satisfy their valencies. The structures were then energy minimized by applying CHARM force field to remove the steric clashes between the atoms in order to get stable conformation.

Active site identification

The binding sites of the receptor proteins were predicted based on ‘receptor cavity method’ using Accelry’s Discovery Studio 4.0. Using this protocol, active sites of the target receptor were identified based upon the inhibitory property of the amino acid residues present in the binding sites.

Ligand preparation and filtration

A collection of 5 phytocompounds from Bael were taken as ligands for docking analysis. The 3D structures of these compounds were downloaded from PubChem database. These ligands were then cleaned up, calculated 3D coordinates and generated ligand conformations by applying ‘prepare ligand protocol’ of Discovery Studio 4.0. After preparation, the compounds were filtered based on the molecular properties for predicting their solubility and permeability in drug discovery. The best known of the physical property filters is Lipinski’s “rule-offive”, which focuses on bioavailability. The rule states that the compounds have molecular mass less than 500 daltons, not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and an octanol-water partition coefficient log P not greater than 5 (Lipinski et al.,2001). The filtered compounds were then used for docking analysis.

Docking

The anti-inflammatory activity of all the 4 phytochemicals reported from Cumin was assessed by docking these compounds against the respective active sites of the target proteins. Discovery studio 4.0 was used in this study to find the interacting compounds of Bael with the selected targets of arthritis. Strategies of Discovery Studio 4.0 are to exhaustively dock or score possible positions of each ligand in the binding site of the proteins. Docking study of the target proteins was done with natural compounds derived from Bael to find the preferred orientation and binding affinity of the compounds with each target protein using scoring functions. A molecular dynamics (MD) simulated-annealing-based algorithm, namely, CDOCKER was used to score the interacting compounds. This method uses a gridbased representation of the protein-ligand potential interactions to calculate the binding affinity (Wu et al., 2003). CDOCKER

uses soft-core potentials, which are found to be effective in the generation of several random conformations of small organics and macromolecules inside the active site of the target protein. Ligands were docked to the proteins followed by scoring them for their relative strength of interaction to identify candidates for drug development. The final poses were then scored based on the total docking energy, which is composed of intramolecular energy of ligand and the ligand-protein interaction. The lowest energy structure was taken as the best fit. Interpretation of the values was done using standards provided by Discovery Studio such as CDOCKER energy, CDOCKER interaction energy, hydrogen bonds, binding energy etc.

Drug likeliness

Drug-likeness is a qualitative concept used in drug design to evaluate how the substance acts like drug with respect to factors like bioavailability. The molecular properties which influence absorption, distribution, metabolism, excretion and toxicity are recognized as a long side therapeutic potency as key determinants of whether a molecule can be successfully developed as a drug (Zhang et al., 2012). These parameters are responsible for about 60 percent failures of all drugs in the clinical phases and so the prediction of ADMET properties plays a significant role in new drug discovery process (Hire et al., 2012). Thus, it has become imperative to design lead compounds which would be easily Gastrically absorbed, easily transported to their targeted site of action, not easily converted into toxic metabolic products and easily eliminated from the body before accumulating in sufficient amounts. The ADMET properties of the compounds were analyzed for drug like candidates.

RESULT AND DISCUSSION

Protein preparation and active site identification

The three dimensional structures of the identified target proteins were retrieved from the protein data bank. PDB ID of the targeted protein structures are mentioned in Table 1.

Ramachandan Plot of the targeted gene

The Ramachandran plot is among the most central concepts in structural biology, seen in publications and textbooks alike. However, with the increasing numbers of known protein structures and greater accuracy of ultra-high resolution protein structures, we are still learning more about the basic principles of protein structure. The use of torsion angles to describe polypeptide and protein conformation was developed by Sasisekharan as part of his studies of the structure of collagen chains during his work as a graduate student in the research group of G.N. Ramachandran. The power of this approach was readily apparent and its use quickly became widespread. Using revised definitions, this so-called Ramachandran plot or ϕ , ψ -plot has remained nearly unchanged in the ensuing fifty years and continues to be an integral tool for protein structure research and education.

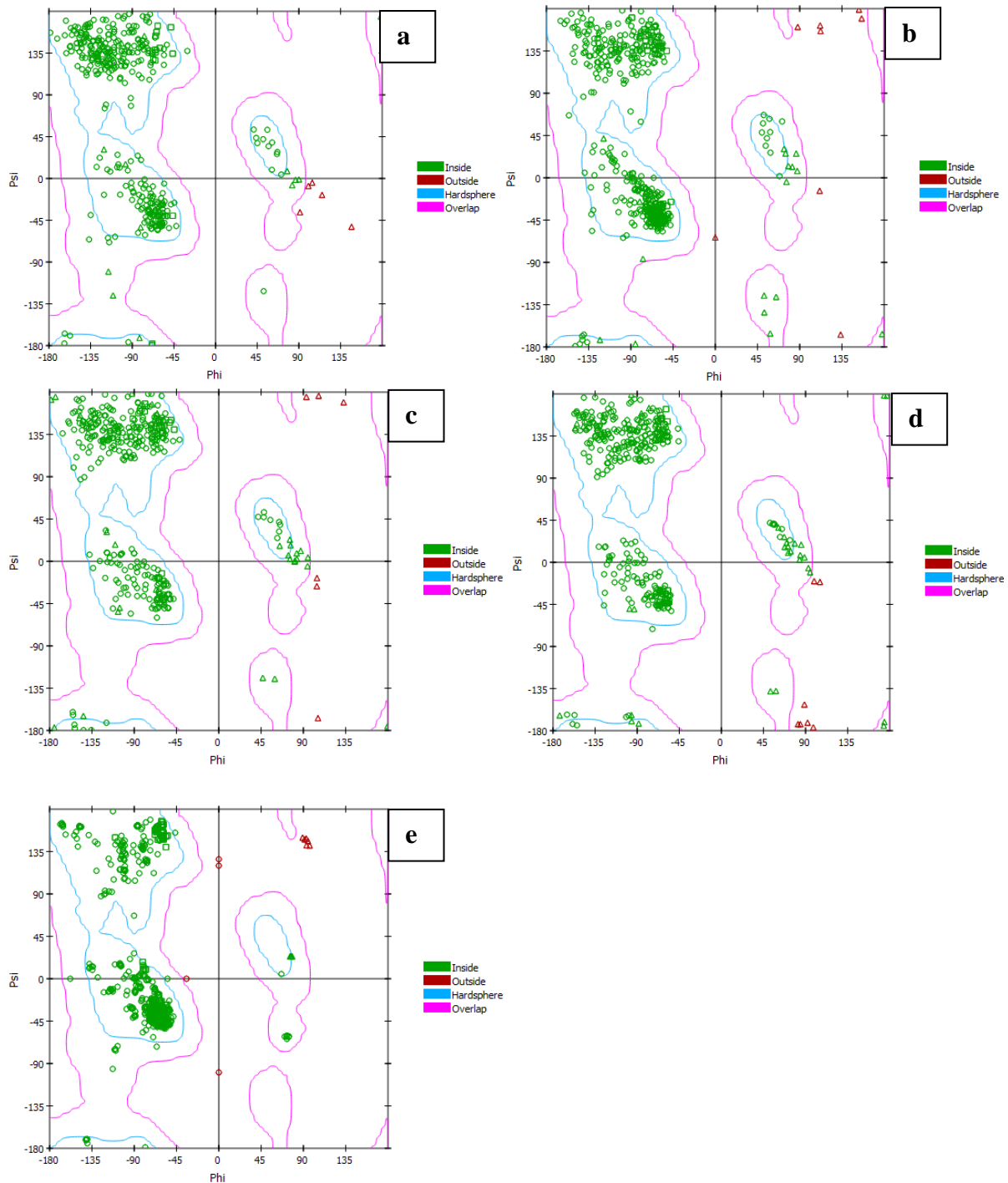


Fig 1: Ramachandran plot of (a) 1HHH (b) 2XWH (c) 3KF2 (d) 3SU4 (e) 5T2P

Hydrophobicity Plot of the Genes:

Protein–protein interactions (protein functionalities) are mediated by water, which compacts individual proteins and promotes close and temporarily stable large-area protein–protein interfaces. In their classic article, Kyte and Doolittle (KD) concluded that the “simplicity and graphic nature of hydrophobicity scales make them very useful tools for the evaluation of protein structures.” In practice, however, attempts to develop hydrophobicity scales (for example, compatible with classical force fields (CFF) in calculating the energetics of protein folding) have encountered many difficulties.

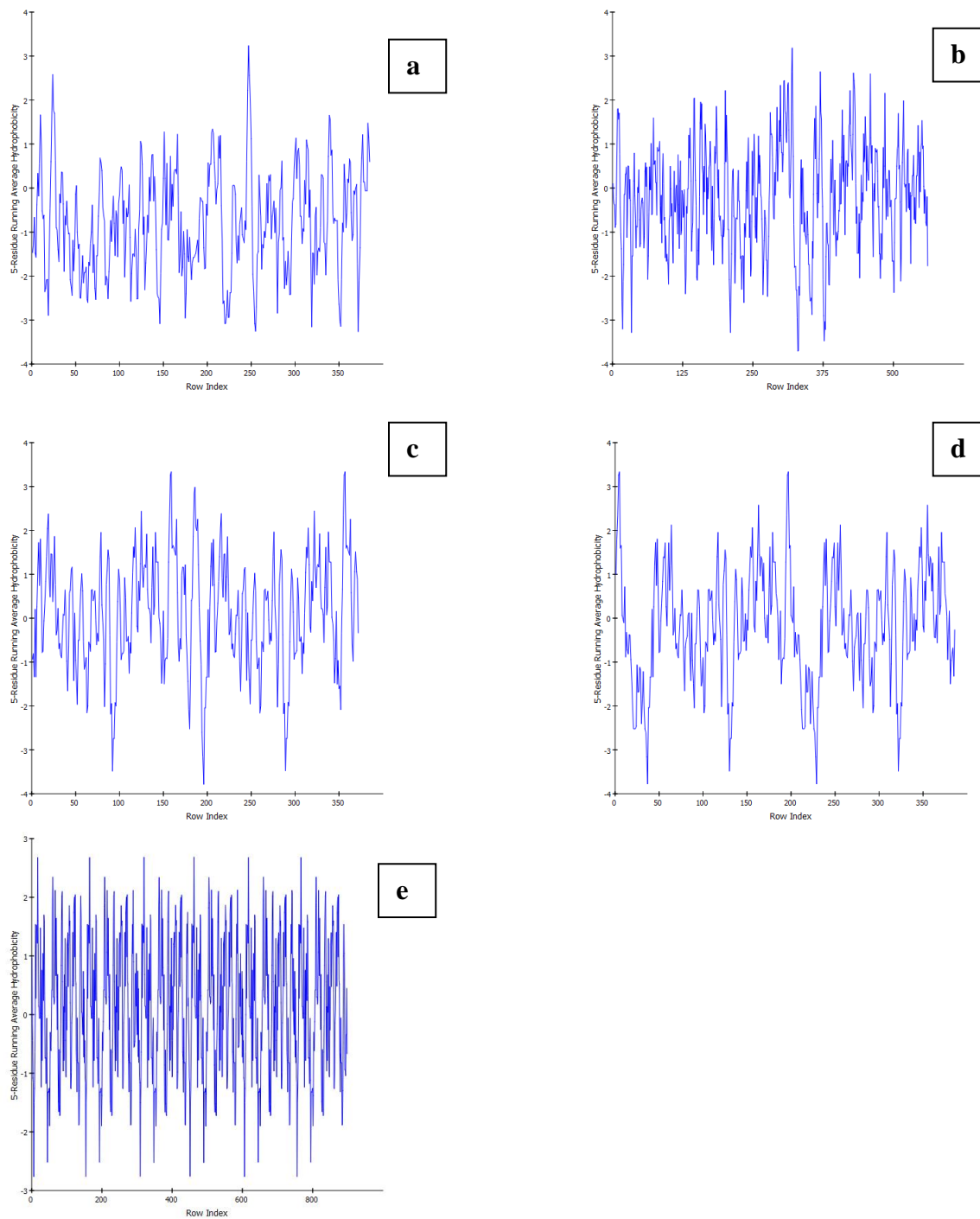


Fig 2: Hydrophobicity Plot of (a) 1HHH (b) 2XWH (c) 3KF2 (d) 3SU4 (e) 5T2P

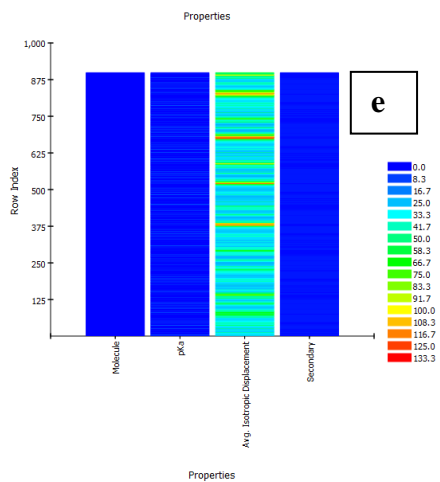
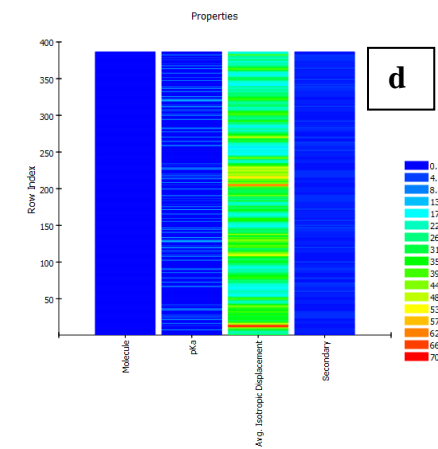
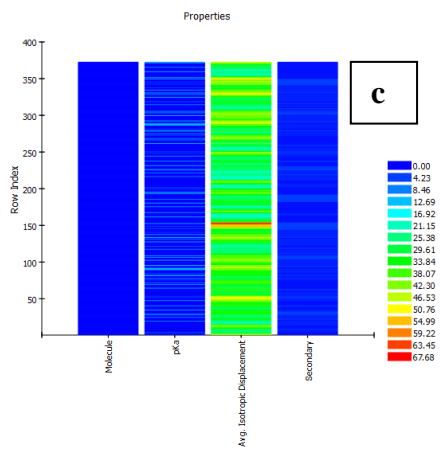
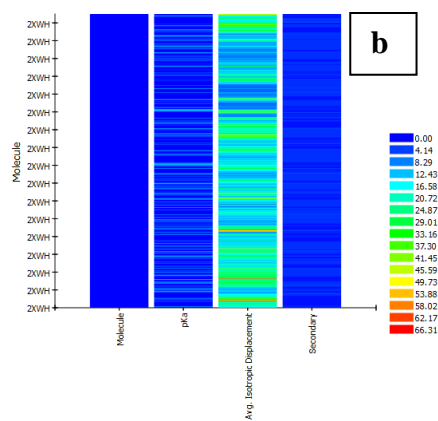
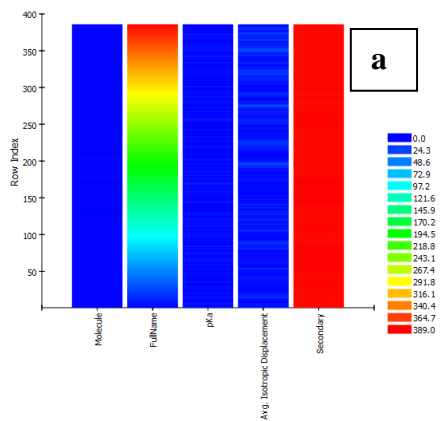


Fig 3: Heat Map Plot of (a) 1HHH (b) 2XWH (c) 3KF2 (d) 3SU4 (e) 5T2P

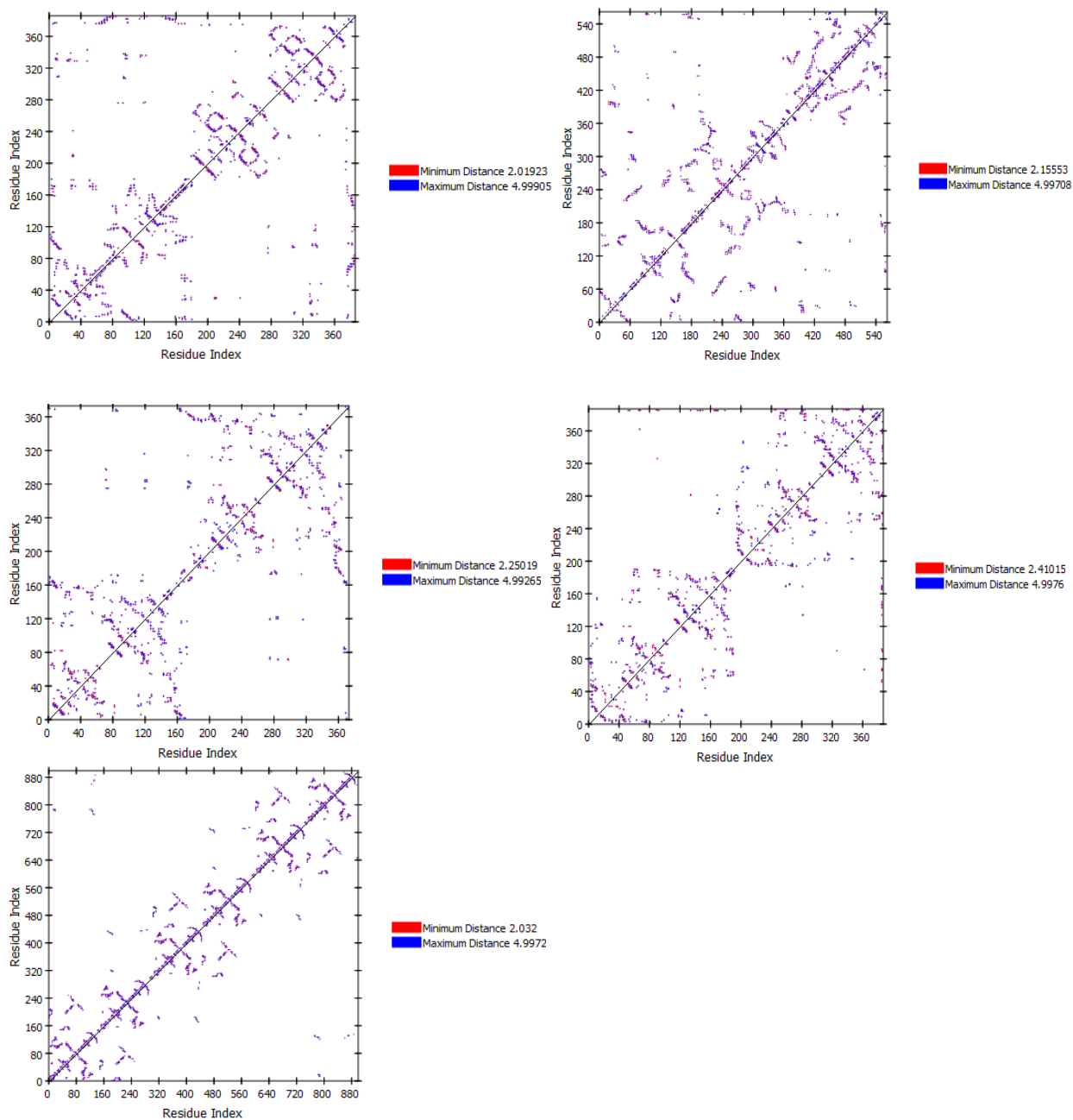


Fig 4: Side chain Plot of (a) 1HHH (b) 2XWH (c) 3KF2 (d) 3SU4 (e) 5T2P

Ligand preparation

4 of the pharmacophores are satisfied Lipinski rule and are expected to be active compounds after Gastric administration. The ligand molecules with least binding energy are considered as compounds with highest binding affinity. This binding affinity indicated a focused interaction between the above compounds with the targets compared to others. The parameters for finding the best inhibitors such as CDOCKER energy, CDOCKER interaction energy and number of hydrogen bonds were also evaluated. CDOCKER energy is the combined energy produced by the sum of internal ligand strain energy and receptor-ligand interaction energy where, CDOCKER interaction energy is the interaction energy between the protein and ligand and the values of these two parameters indicate the strength of interaction between the proteins and the ligands. Besides least binding energy, compounds with least atomic energy difference between

CDOCKER energy and CDOCKER interaction energy were analyzed. Based on CDOCKER energy and CDOCKER interaction energy, Fig 5 is showing the result.

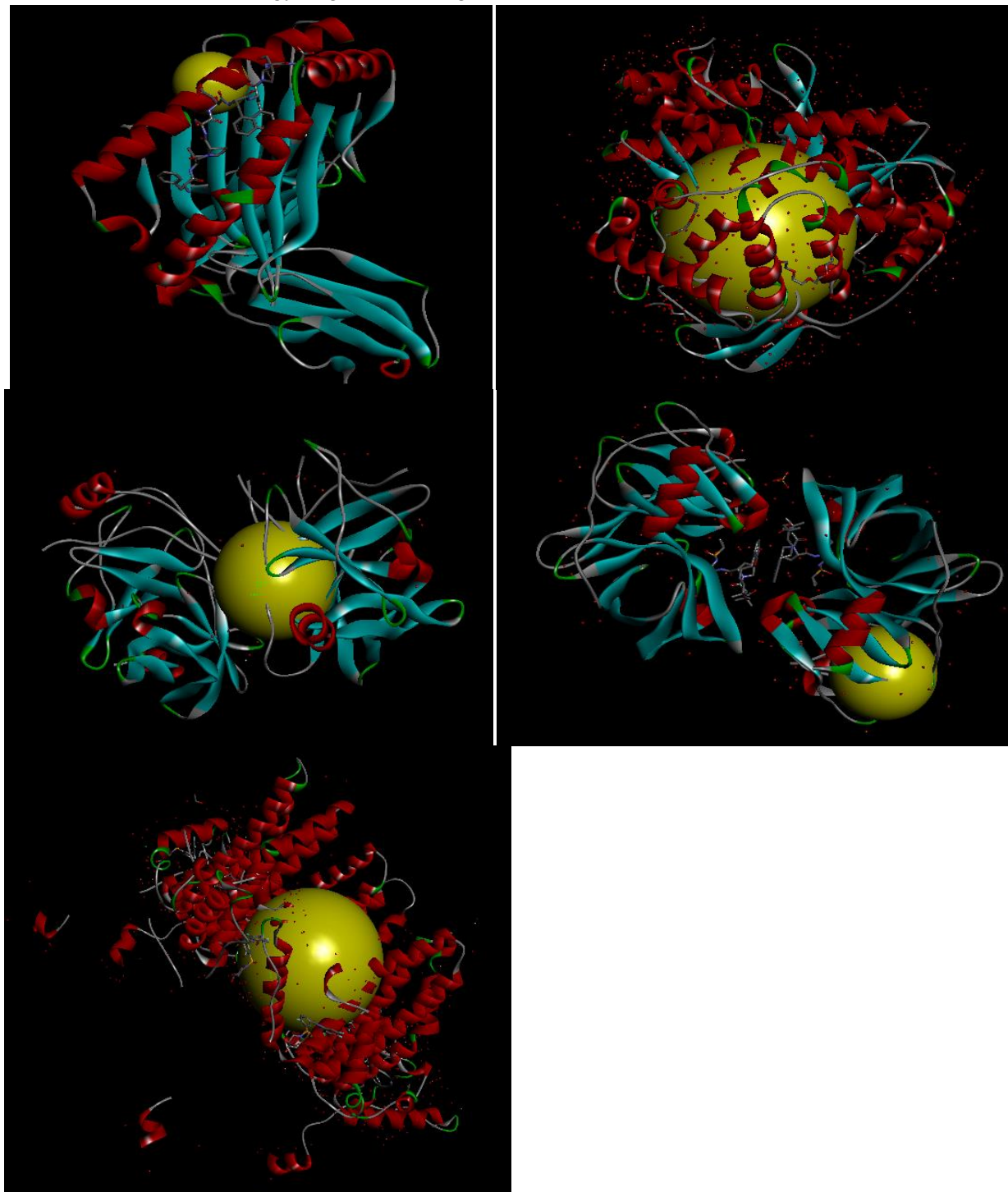


Fig 5: Docking Result of (a) 1HHH (b) 2XWH (c) 3KF2 (d) 3SU4 (e) 5T2P ADMET Evaluation

ADMET analysis report

Considering the comparable CDOCKER energy, interaction energy and binding energy, three compounds were forwarded for ADMET analysis. These studies are based on the ADMET (Absorption, Distribution,

Metabolism, Excretion and Toxicity) properties of the compounds. These properties provide insights in to the pharmacokinetic properties of the compounds and were checked using Discovery Studio's built in ADMET protocol. The various parameters tested in this study were aqueous solubility, Blood Brain Barrier (BBB) level, Hepatotoxicity, Absorption level, AlogP and CYPD26. Pharmacokinetic properties of the best fit phytochemicals showed that two of the compounds had passed all the pharmacokinetic parameters. The compounds that passed the parameters were N-methyltyramine and dalbergioidin. These compounds were thus selected as the best compounds in this study as they had good interaction scores along with ADMET properties.

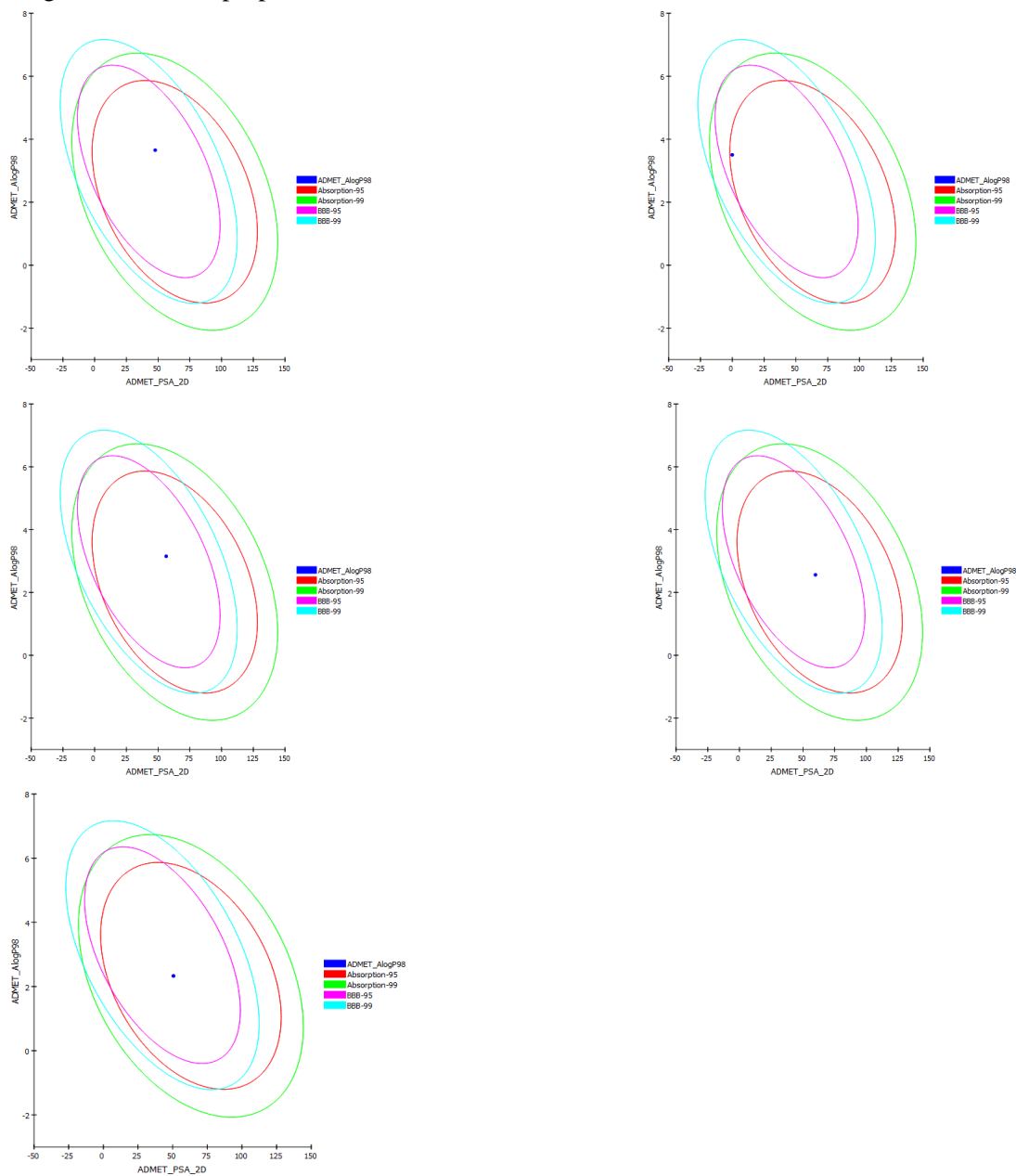


Fig 6: ADMET test analysis report

CONCLUSION

The identified pharmacophores can be isolated from the Bael and can be commercialized as the natural drug for the Hepatitis Gene which is having lesser harmful side effect from the chemotherapeutic drug available in the market. This drug will also be very cheaper from the available drugs and these drugs are also not harmful for the normal cells as they are derived from the natural products.

The unique feature of the study is to targeted gene therapy for a particular cancer. This will help our future medicine to be completely allied to the Pharmacophores and the uses of synthetic and carcinogenic drug will reduce.

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