In-silico Analysis of Effects of Methi Extract on Animal Disease Gestatinal Diabetes

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ABSTRACT

Antiquated India is one of the pioneers of investigations of plants as medication, for example Ayurveda. In our social and monetary life we barely deal with our food we are taking. One such novel herb is Methi which has frequently been viewed as a mind supporter. The entire plant including the blossoms can be utilized for restorative purposes. It has a harsh and sweet taste and is known to give a cooling vitality. Methi is loaded with cancer prevention agents that are basic for carrying on with a sound life. We are utilizing this property of Methi to get some new medications for Aster Yellow. The employments of different pesticides, additives, and so on transform the nourishments into poison. Also the reactions of these pesticides and additives, and so on are perilous as on the grounds that it prompts commencement of various malignant growth. In this entire world, the quantity of patients kicking the bucket from malignant growth is expanding in a compromising manner. In-silico investigation has done utilizing programming and we further focused on a portion of the qualities answerable for Aster Yellow and pharmacophores from Methi and destroyed some silico examination. In this we have discovered that these two pharmacophores are having better Mol Doc score from any others.

KEY WORDS: METHI, DOCKING, *IN SILICO* ANALYSIS, GESTATIONAL DIABETES, PHARMACOPHORE

INTRODUCTION

Plants made due for many years on planet earth by constantly developing and adjusting. Prescriptions acquired from Mother Nature, particularly got from plants, have been all around archived for quite a long while . Indeed, even today, as indicated by World Health Organization, about 80% of total populace from the creating and immature nations despite everything rely upon plant-inferred prescriptions for their human services prerequisites. Fenugreek (*Trigonella foenum-graecum Linn*.), is a shortliving yearly plant, has a place with the *Fabaceae* family. It is developed in numerous pieces of Asia, Africa, and Europe as food, condiment, zest, and as local medication. The variety *Trigonella* is named concerning its triangular formed blossoms, and in Latin little triangle is alluded to as *Trigonella*. The species *foenum-graecum* gets its name from verifiable point of view of Romans, since it is utilized as regular yield grub for creatures in Greece . Fenugreek plant achieves the stature of 1–2 feet and bears green trifoliate leaves. The blossoms are white to yellow in shading and the plant conveys slight cases. The units are around 15 cm long and they contain on a normal 10–20 seeds. Fenugreek seeds are brilliant yellow in shading and their normal stature, width, and thickness are 4.01–4.19, 2.35–2.60, and 2.40–2.66 mm, separately. Fenugreek seeds are the most significant and all around concentrated piece of fenugreek plant. The dried fenugreek seeds are grounded to acquire fenugreek seed powder which is utilized as fixing. Fenugreek gum is acquired from the endosperm of the seeds. Fenugreek is utilized as a zest and herb in numerous culinary dishes and its green leaves used to enhance dishes or eaten as greens and seeds are utilized for seasonings or squashed to get ready curry powders and glues' utilized for seasonings or squashed to plan curry powders and glue. Notwithstanding being utilized in different food arrangements, fenugreek likewise has recuperating benefits. Fenugreek is one of the most seasoned restorative plant and the therapeutic properties are very much reported in the old clinical writing. In Ayurveda, the customary Indian clinical framework, fenugreek was utilized as a stomach related guide and old Egyptians utilized it as lactation help . In customary Chinese medication, fenugreek was utilized to treat edema in the legs. There are number of employments of fenugreek, including the treatment of lung blockage and sinus, heartburn, sparseness in men, hair tonic and conditioner and as galactogogue. As of now, an enormous number of studies have revealed positive insight into fenugreek's therapeutic properties, for example, antioxidant , anti-inflammatory, antidiabetic, antiobesity, anticancer , hepatoprotective , anti-hyperlipidemic ladies' wellbeing and sexual health modulating activities. All These disease avoidance properties of fenugreek are because of essence of various exhibits of phytochemicals and their distinctive diverse pharmacological and organic exercises. In light of all these assortment of restorative properties, Fenugreek is picked as a therapeutic plant for this work with the goal that it might be useful to fix some disease.

During pregnancy, the body produces a larger amount of certain hormones that impact the placenta, and help to maintain a healthy pregnancy. This increase in hormones leads to insulin resistance, which increases the amount of glucose in the blood stream. This is normal in pregnancy, as this extra glucose is needed to support the baby. However, when insulin resistance becomes too great, and the amount of glucose in the bloodstream is very high, gestational diabetes can result .It is a form of high blood sugar affecting pregnant women. Those who develop gestational diabetes are at higher risk of developing type 2 diabetes later in life.More than 1 million cases of gestatinal diabetes found in India per year. During pregnancy, certain hormones such as chorionic gonadotropin (hCG), estrogen, and progesterone are released that can lead to a mass amount of glucose in the blood. In addition, pregnancy hormones like placental lactogen can interfere with susceptible insulin receptors, which further increases blood glucose levels. When the amount of insulin produced is less than the amount needed to handle blood glucose levels, gestational diabetes can arise .Gestational diabetis can cause birth injury.

Materials and methods

Various pharmacophores of Methi leaves have been listed and their respective SDF were taken accordingly from Pubchem, Molinstincts, and Chebi. The enzyme corresponding to microbe of Aster Yellow has 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 Aster Yellow

Sl.No	Pharmacophores from Methi	Targeted Animal Gene (Gestational Diabetes)	PDB No of the Genes
1	Trigonelline	Adiponectin, C1q And Collagen Domain Containing	6U6N
2	Methyl Coumarin	Peroxisome Proliferator Activated Receptor Gamma	6ONI
3	Carpaine	C-Reactive Protein	6NMT
4	Choline	Leptin	6E2P

Protein identification and preparation

The reported molecular targets responsible for Rice Tungro Gene 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 Cumin 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 Cumin 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 Cumin 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 Gastricly 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 structure 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 proteinstructures 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.

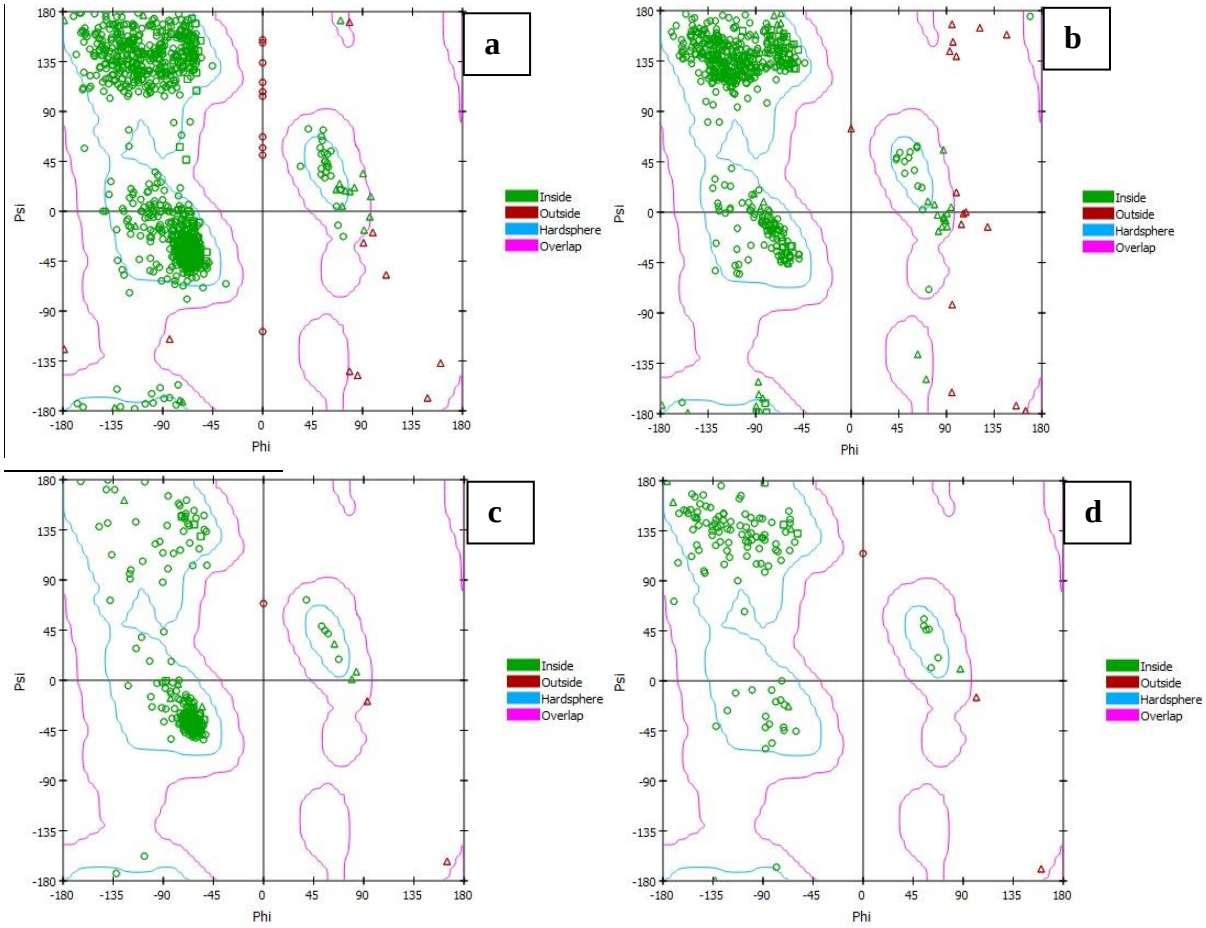
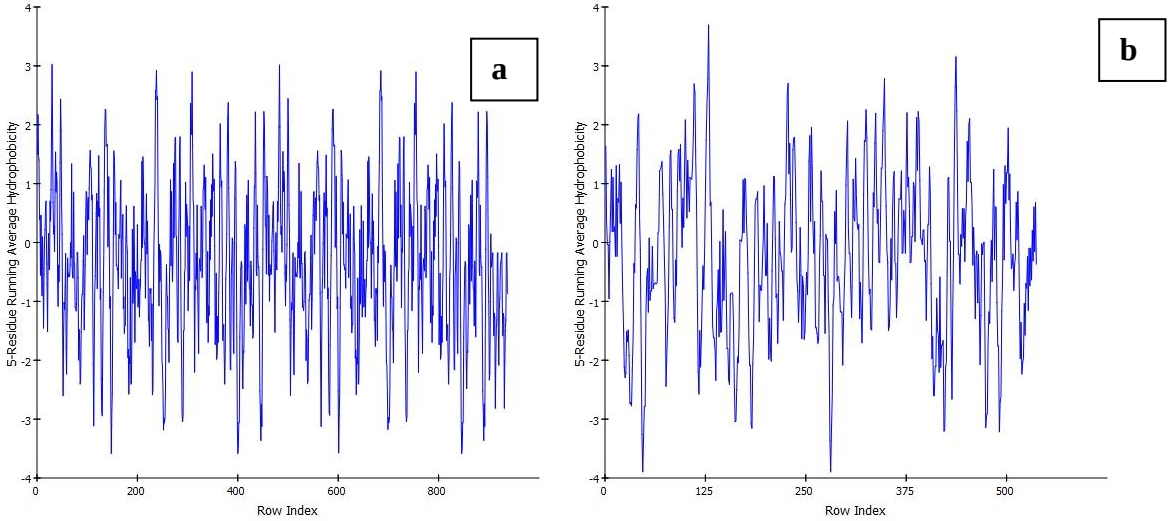


Fig 1: Ramachandan plot of (a) 6E2P (b) 6NMT (c) 6ONI (d) 6U6N

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



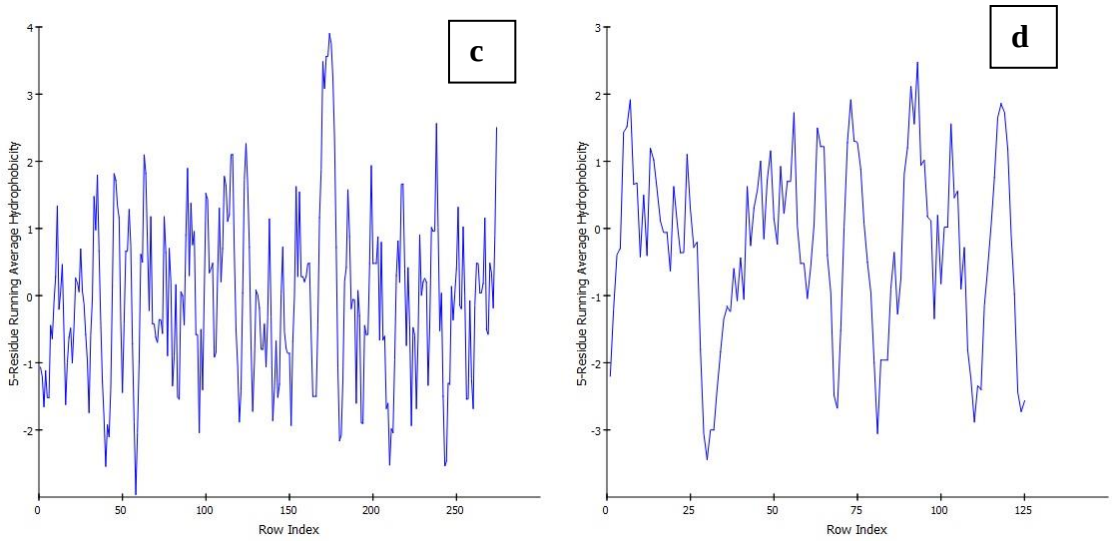
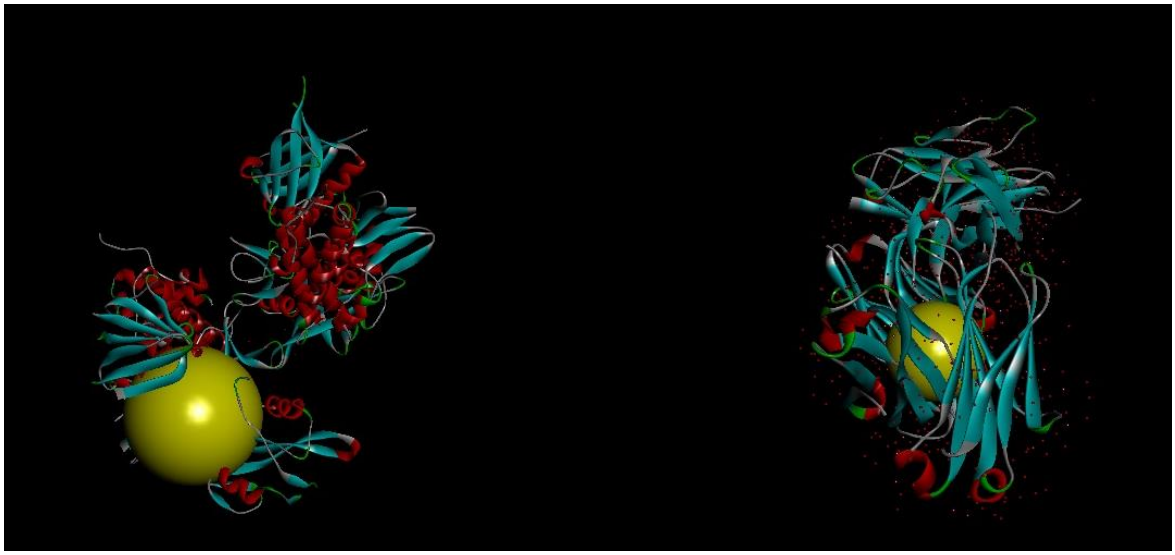


Fig 2: Hydrophobicity Plot of (a) 6E2P (b) 6NMT (c) 6ONI (d) 6U6N

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 4 is showing the result.



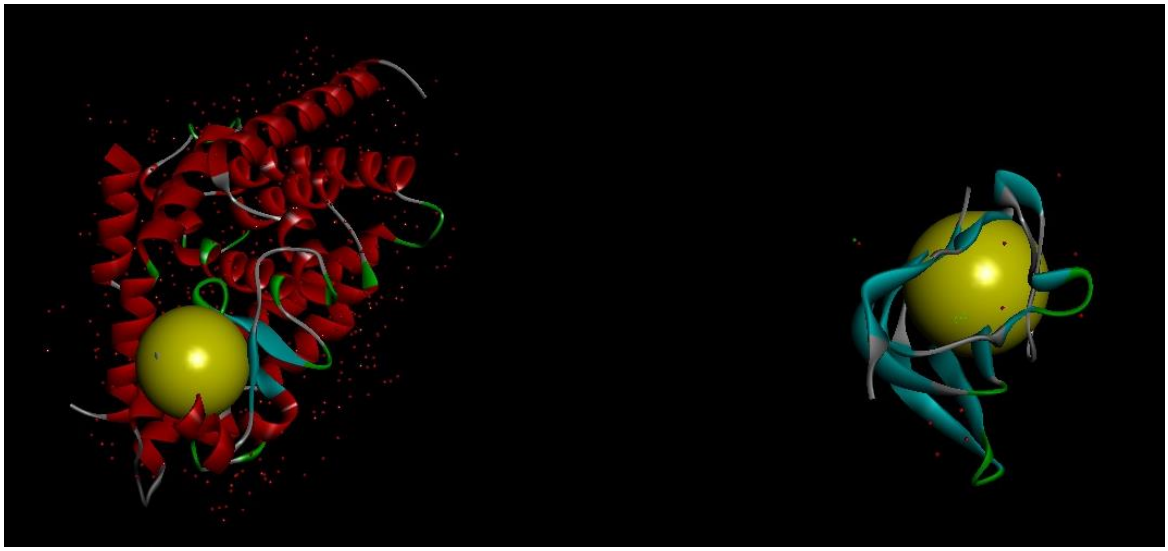


Fig 3: Docking Result of (a) 6E2P (b) 6NMT (c) 6ONI (d) 6U6N

ADMET Evaluation

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 pharamacokinetic 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.

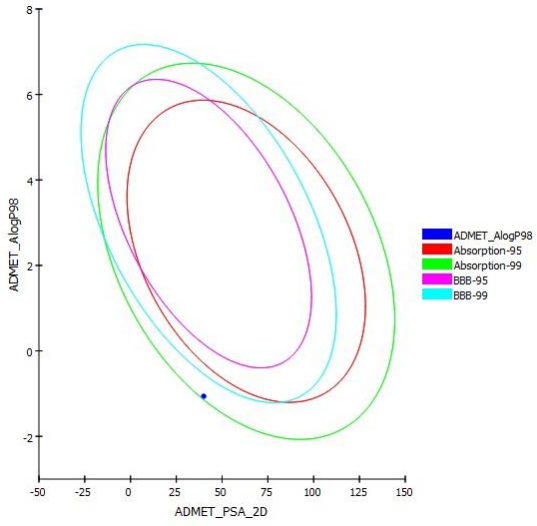


Fig 4: ADMET test analysis report

CONCLUSION

The identified pharmacophores can be isolated from the Cumin and can be commercialized as the natural drug for the gestatinal diabetes 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 Pharmachophores and the uses of synthetic and carcinogenic drug will reduce.

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