In-silico Analysis of Effects Of Stevia Extract as Biopesticides on Leaf Blight

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ABSTRACT

Due to the ever-increasing worldwide plantation of sweet leaf Stevia rebaudiana (Bertoni), how to efficiently and effectively utilize the huge amounts of leaf residues that contain abundant nutrients after sweetener extraction becomes an eminent issue. One option is to return these residues into soil, as organic manure in the fresh or composted form, in order to both sustain soil fertility and avoid potential environmental pollution. In a field experiment, we studied if the Stevia leaf residue returning affected both plant and soil microbial growths as well as the possible change of soil microbial community composition. We have identified some molecules and plant diseases and have performed molecular docking and we got some good data to carry out our work further

KEY WORDS: BIOPESTICIDES, LEAF BLIGHT, MOLECULAR DOCKING, PHARMACOPHORE, STEVIA

INTRODUCTION

Approximately 80% of the world populations depend exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual however in the recent years, more and more people are complementing their treatment with natural supplements (Dursum et al., 2004). Nowadays motivation of people towards herbs is increasing due to the concern about the side effects of synthetic chemical drugs. People want to concern their own health rather than submitting themselves to impersonal health care system. Many herbal and some commonmedicinal plants are good sources of antioxidant compounds. Many of the biologically active substances found in plants, including phenolic compounds (flavonoid, phenolics) are known to possess potential antioxidant properties. The antioxidant activity of medicinal plants depends on the concentration of individual antioxidant entering into the composition (Larson, 1988). Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals. Free radicals have been implicated in the etiology of several major human ailments including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis (Devasagayam et al., 2004). Antiox- idants have been reported to prevent oxidative damage caused by free radical, they can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers (Buyukokuroglu et al., 2001). The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body which are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids lipoproteins and DNA (Farber, 1994). This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases as well as ageing process. Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Natural plant-based antioxidants especially phenolics and flavonoids have been exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu et al., 2000). Stevia rebuadiana (Bert.), Bertoni is an herbaceous perennial plant of the Asteraceae family. It is native of Paraguay, where it grows wild in sandy soil (Goenadi, 1983). The main sweet component in the leaves of S. rebuadiana is stevioside (Geuns, 2000). Stevia sweetener extractives have been suggested to exert beneficial effects on human health, including antihypertensive (Chan et al., 2000), antihyperglycemic noncariogenic, anti human rota virus activities, glucose metabolism (Suanarunsawat and Chaiyabutr, 1997) and renal function (Jutabha and Chatsudthipong, 2000). Aqueous extract of S. rebuadiana dried leaves induce systemic and renal vasodilation, causing hypotension, diuresis and natriuresis in rats (Melis, 1995).

MATERIALS and METHODS

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

Sl.No	Stevia Pharmacophores	Targeted Plant Gene Leaf Blight	PDB No of the Genes
1	Palmitic acid	IMMUNE SYSTEM	5H3C
2	Stevioside	OXIDOREDUCTASE	5ZF2
3	Steviol	CELL CYCLE	2QI2
4	Dulcoside A		

Protein identification and preparation

The reported molecular targets responsible for 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 Stevia 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 Stevia 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 Stevia 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 Stevia 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.

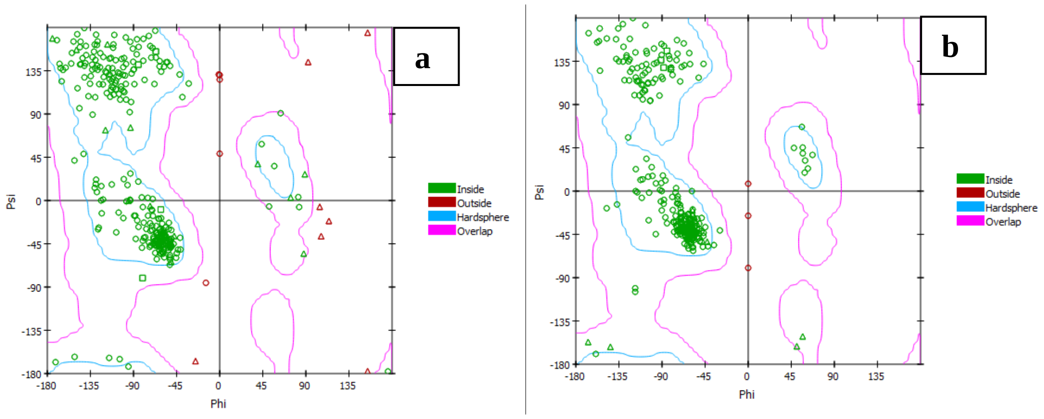
RESULT AND DISCUSSION

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.



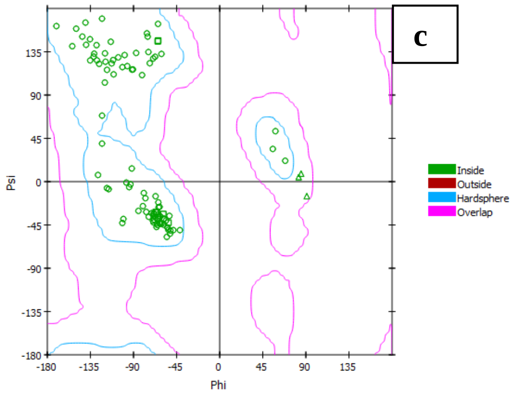
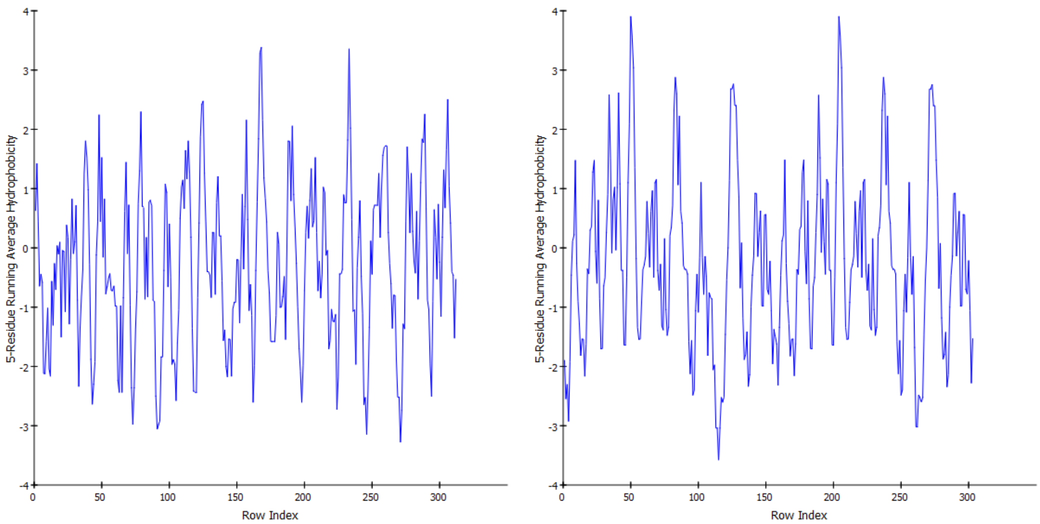


Fig 1: Ramachandan Plot of (a) 2QI2, (b) 5H3C, (c) 5ZF2

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

a **b**



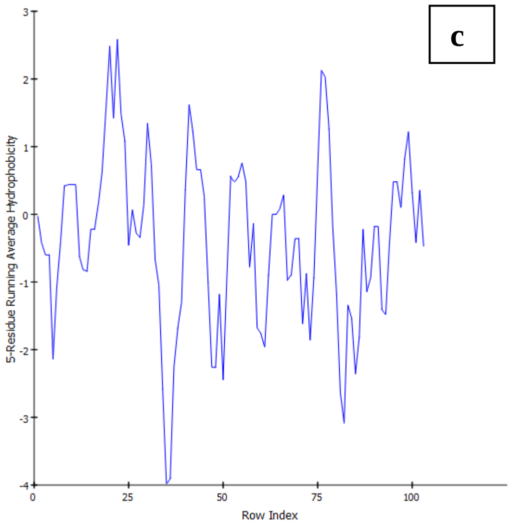


Fig 2: Hydrophobicity plot of (a) 2QI2, (b) 5H3C, (c) 5ZF2

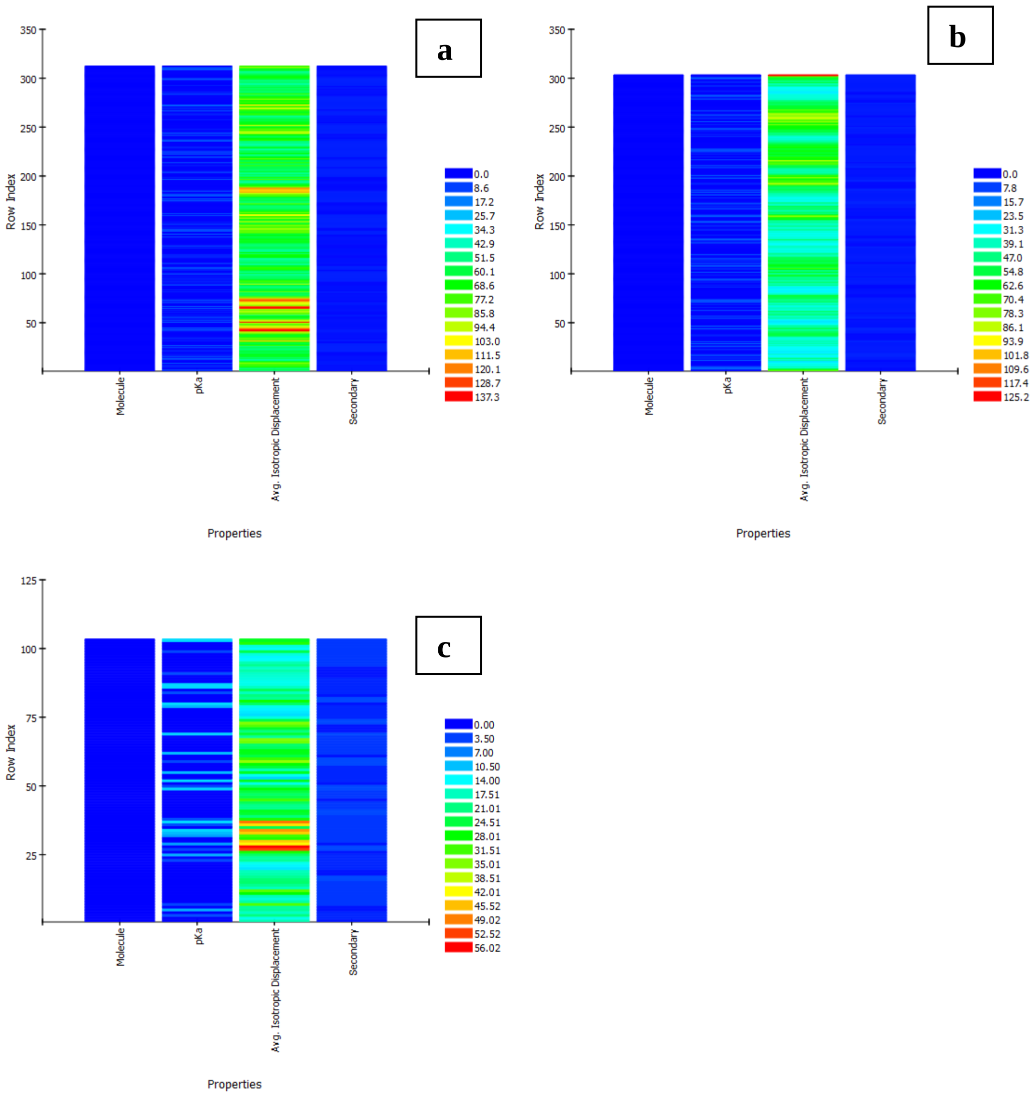


Fig 3: Heatmap of (a) 2QI2, (b) 5H3C, (c) 5ZF2

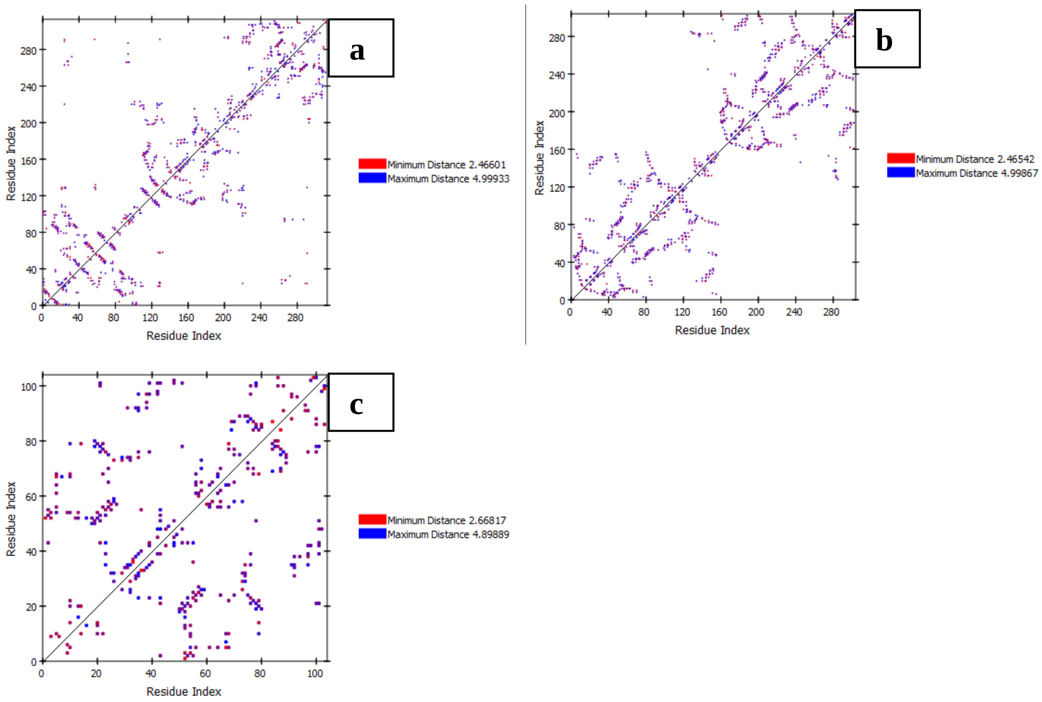
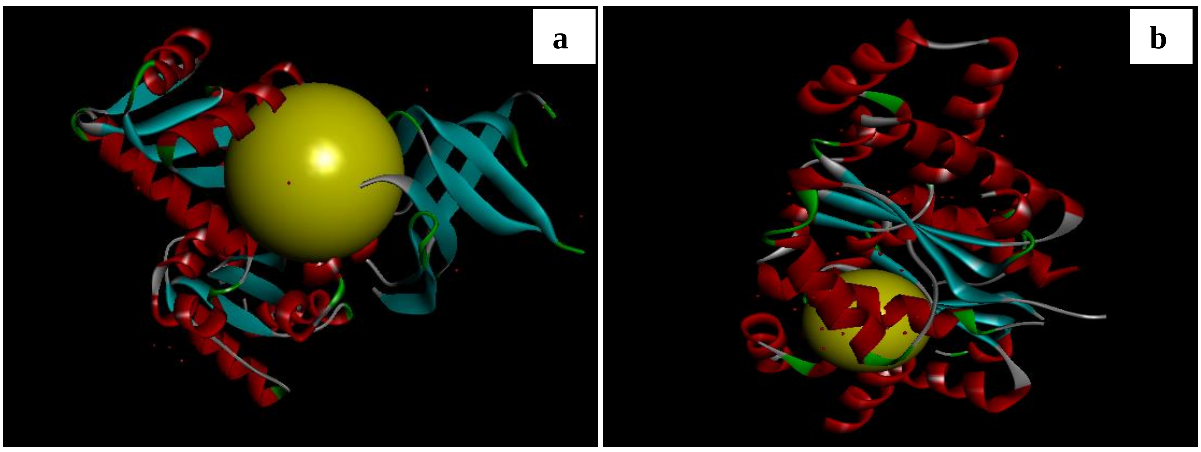


Fig 4: Side chain analysis report of (a) 2QI2, (b) 5H3C, (c) 5ZF2

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.



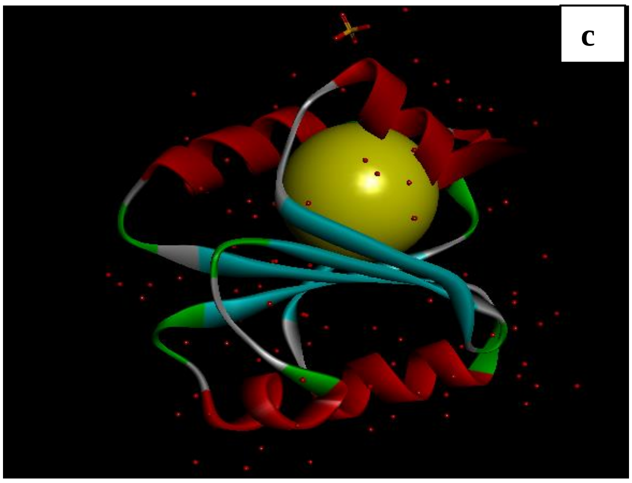
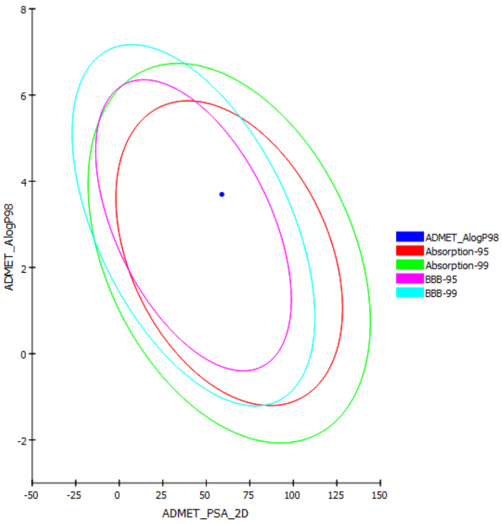
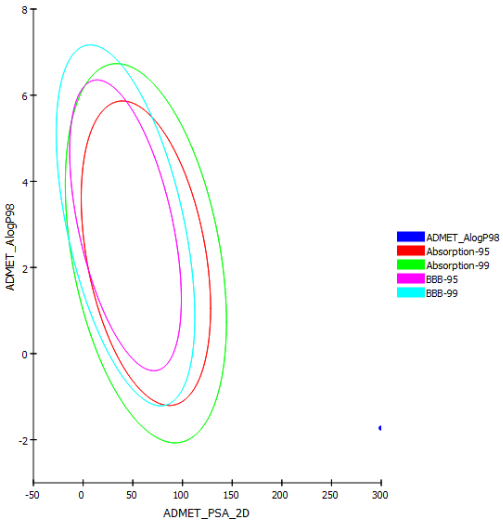


Fig 5: Docking analysis report of (a) 2QI2, (b) 5H3C, (c) 5ZF2

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.





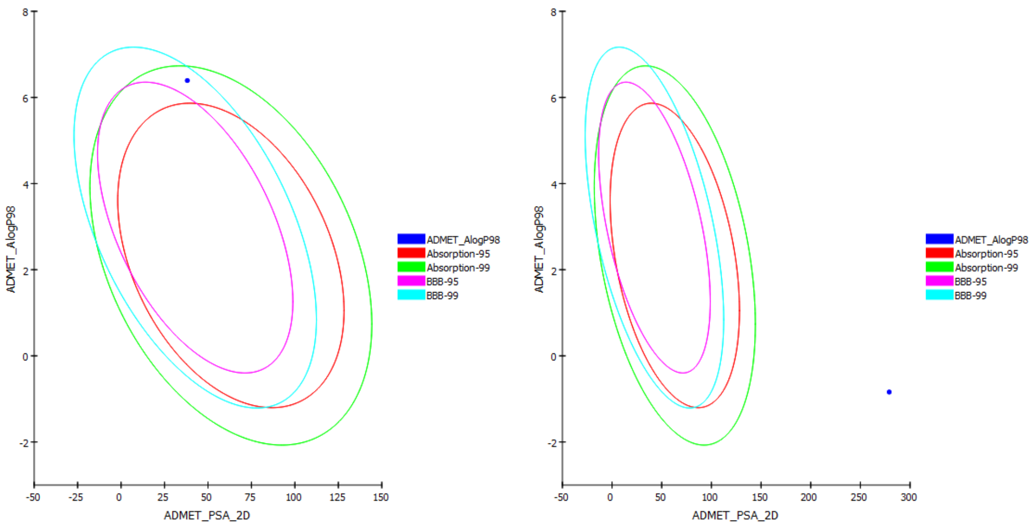


Fig 6: ADMET analysis report

CONCLUSION

The identified pharmacophores can be isolated from the Stevia and can be commercialized as the natural drug for the Leaf Blight 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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