# *In-silico* Analysis of Effects Of Ajwain Extract on Plant Disease

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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 Ajwain 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. Ajwain is full of antioxidants that are essential for living a healthy life. We are using this property of Ajwain to get some new drugs for Aster Yellow. 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 Aster Yellow and pharmacophores from Ajwain 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: AJWAIN, DOCKING, *IN SILICO* ANALYSIS, ASTER YELLOW, PHARMACOPHORE**

## INTRODUCTION

Ayurveda is the oldest medicine system originates in the Indian subcontinent about 5000 years ago. In Ayurved herbs play important role. “Trachyspermum-ammi” (T.ammi) which is popularly known as “Ajwain” is an annual herb in the family of Umelliferae or Apiaceae. It is also known as bishop’s weed or carom. “Ajmoda” is the Sanskrit name of Ajwain this generally cultivated in Iran and India especially in arid and semi-arid region. According to Ayruveda, Ajwain is a powerful cleanser. It is helpful for stimulating the apatite and enhancing digestion. It is recommended to help alleviate gas and discomfort in the stomach. It is easy also helpful for the functioning of the respiratory system and the kidney. The aromatic plant Ajwain is an annual herbaceous. It height can extend to 30 cm to 70 cm. They are having feathery leaves and red flowers. When the seed are ready to cultivate they are dried and threshed. These seeds are grayish-green in colour with striped and curved. They tastes hot and bitter with leaving the tongue numb for a second. The major oil found in ajwain is Thymol which is strong germicide,anti-spasmodic and fungicide. It contains health benefiting oils like cymene, gamma- terepenine, lupeol, linoeric acid, stearic acid, etc.It is also contain moisture, protein, fat, mineral, fiber, carbohydrates, calcium, etc. The chemical extracted from bishop weeds has antibacterial, antifungal, antitussive,anti-inflammatory,and analgesic effects as well as antioxidant, and antitumor in nature. So, we are trying examining the herb’s essential oil and their behavior against hospital-acquired pathogens.

## Materials and methods

Various pharmacophores of Ajwain 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 Ajwain | Targeted Genes from Aster Yellow | PDB No of the Genes |
|---|---|---|---|
| **1** | Thymol | DEAD-box ATP-dependent RNA helicase 10-like | 5GI4 |
| **2** | Lupeol | protein CHROMATIN REMODELING 35-like | 5IKF |
| **3** | linoleic acid | ATP-dependent DNA helicase homolog RECG, chloroplastic-like | 2KYY |
| **4** | Gamma terpinene | | |

## 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.

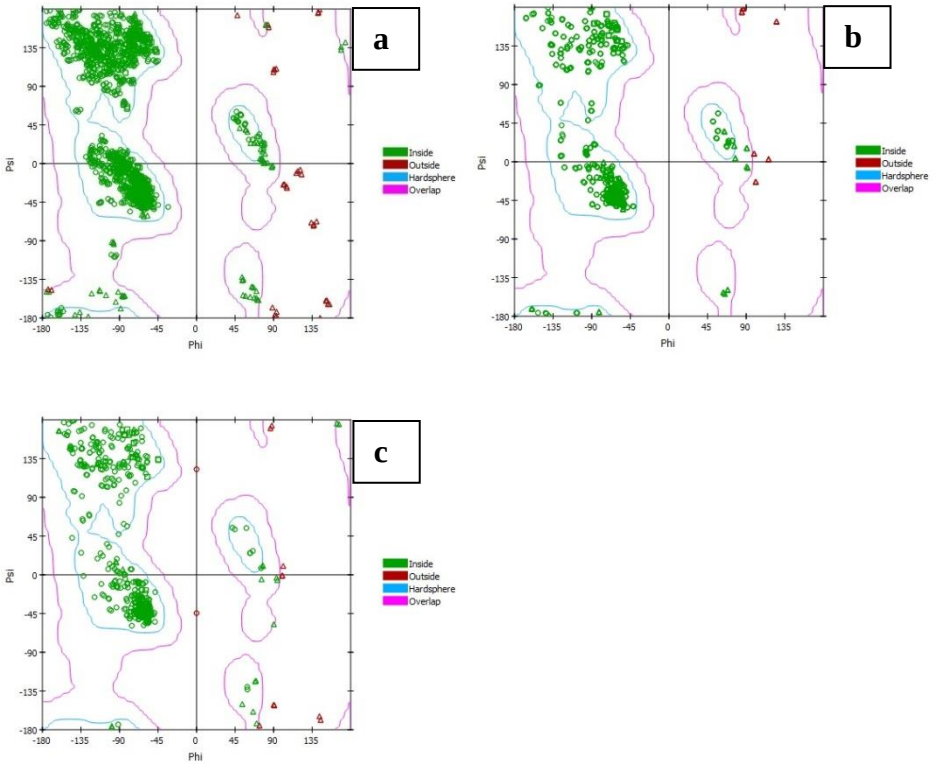



**Fig 1: Ramachandan plot of (a) 5GI4 (b) 5IKF (c) 2KYY**

**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.

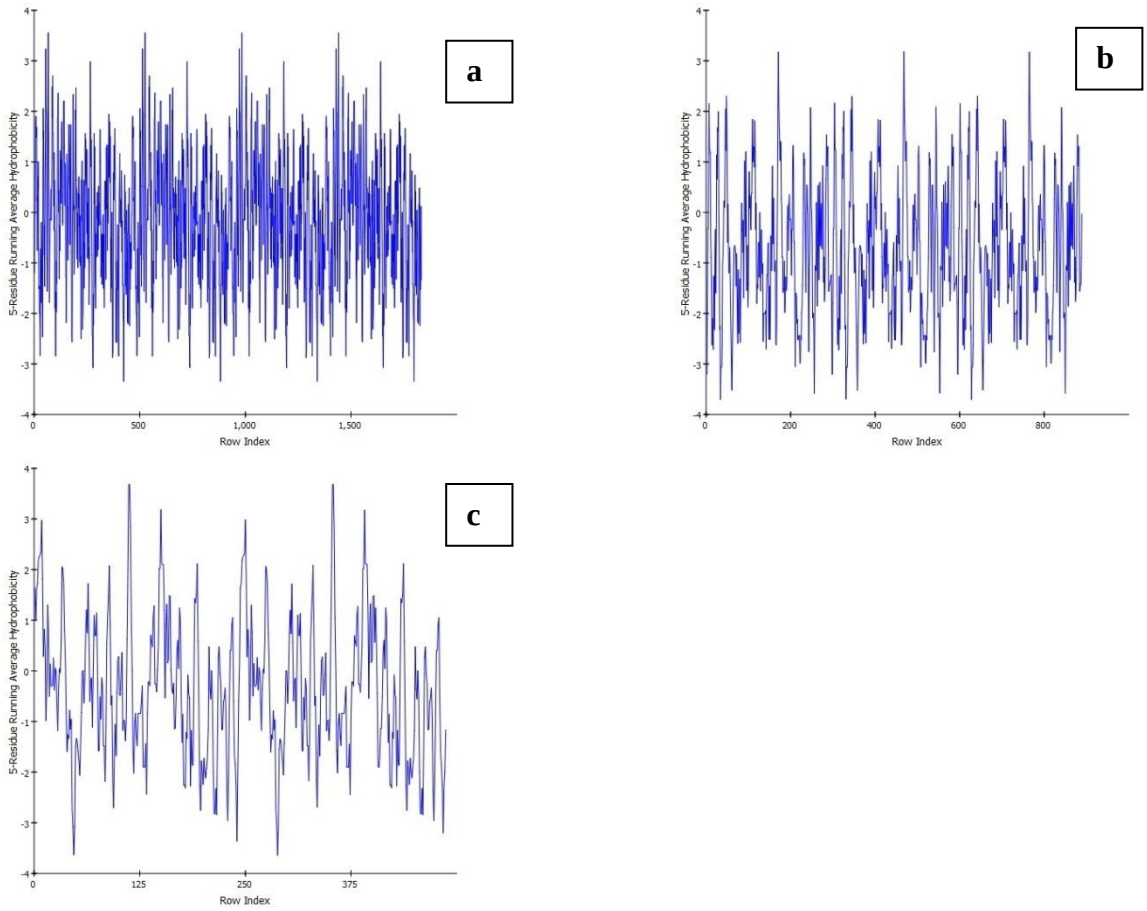
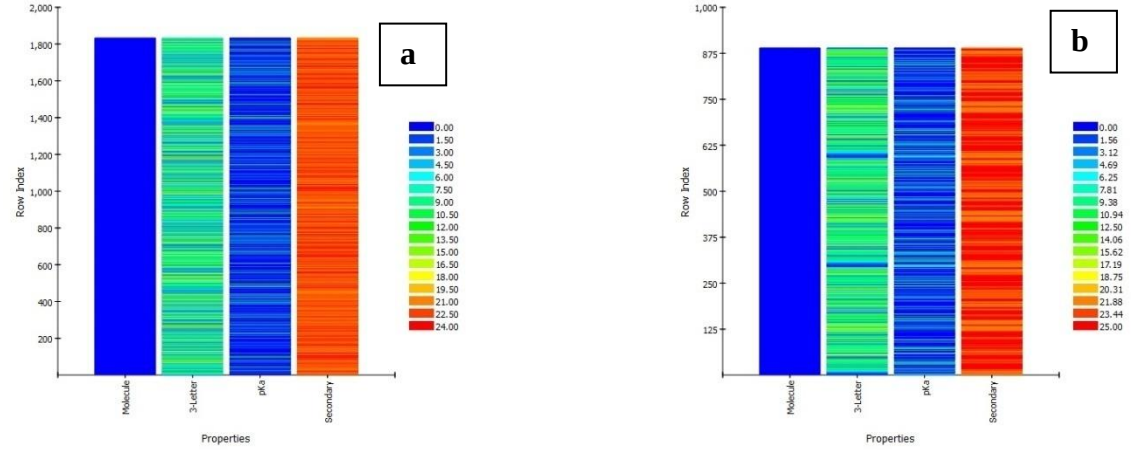



**Fig 2: Hydrophobicity Plot of (a) 5GI4 (b) 5IKF (c) 2KYY**



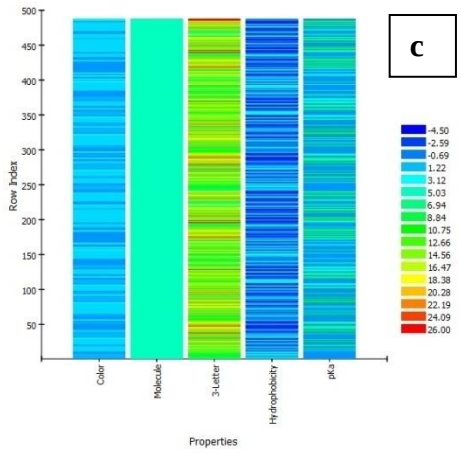



**Fig 3: Heat Map Plot of (a) 5GI4 (b) 5IKF (c) 2KYY**

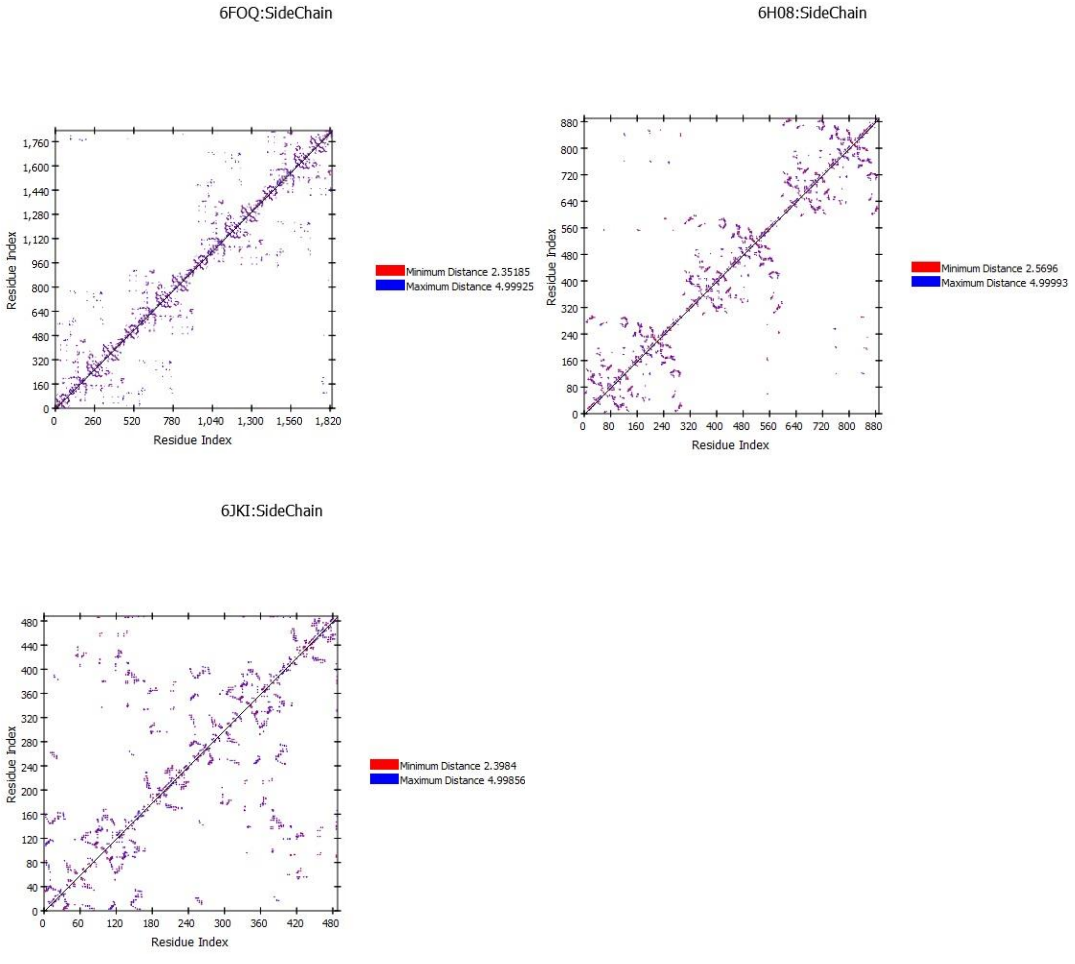



**Fig 4: Side chain Plot of (a) 5GI4 (b) 5IKF (c) 2KYY**

**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.

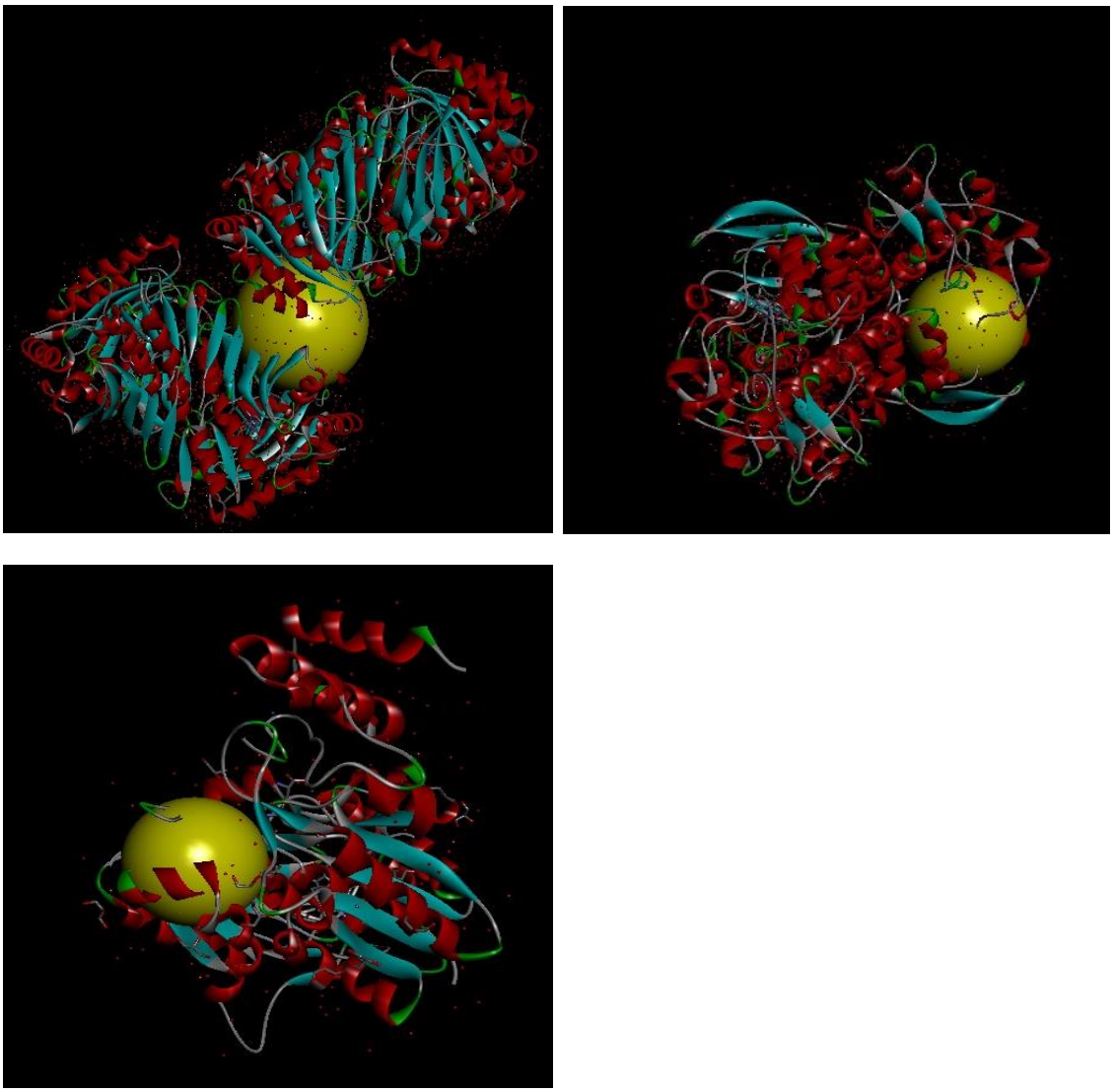


**Fig 5: Docking Result of (a) 5GI4 (b) 5IKF (c) 2KYY**

**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.

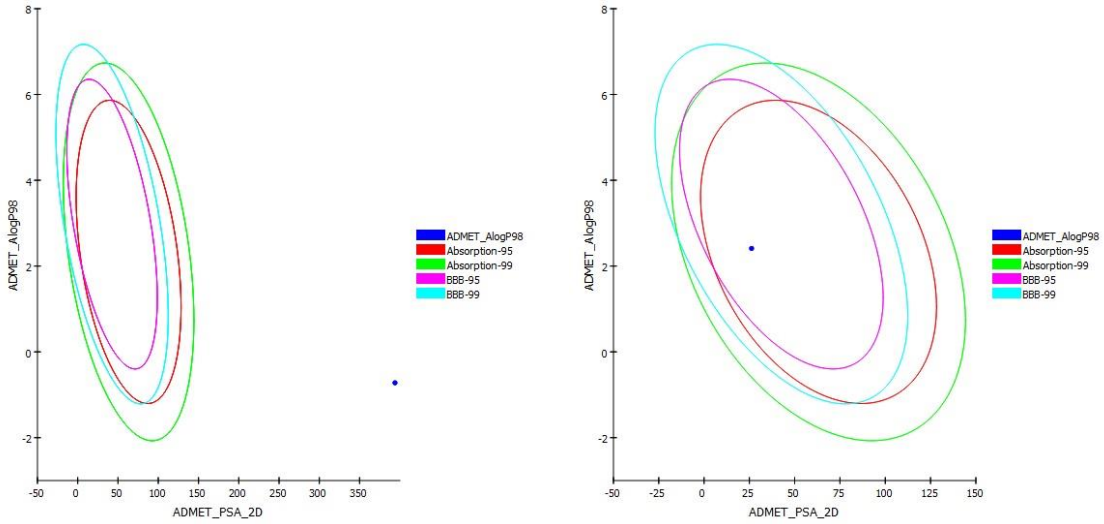



**Fig 6: ADMET test analysis report**

## CONCLUSION

The identified pharmacophores can be isolated from the Cumin and can be commercialized as the natural drug for the Rice Tungro 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.

**References:**

Abdolhamid B, Ebrahimabadi AH, Asma M, Mohsen B, Fereshteh JK, Hossein B, Antioxidant and antimicrobial activity evaluation and essential oil analysis of Semenovia tragioides Boiss from Iran,Food Chem, 122, 2010, 553–558.

Donglu, Zhang, Gang, Luo, Xinxin, Ding and Chuang, Lu. 2012. Preclinical experimental models of drug metabolism and disposition in drug discovery and development. Acta. Pharm. Sin.B., 2 (6):549-561.

Jeet K, Devi N, Thakur N, Tomar S, Shalta L, Thakur R. Trachyspermum ammi: A comprehensive review. Int Res J Pharm 2012;3:133-138 Indian Spices-Ajwain2013.

Khan, R., Adil, M., Danishuddin, M., Verma, P. K., & Khan, A. U. (2012). In vitro and in vivo inhibition of Streptococcus mutans biofilm by Trachyspermum ammi seeds: an approach of alternative medicine, Phytomedicine,19(8), 747-755.30.

Heath HB, Source Book of Flavours, Westport, Avi, 1981, 890.

Hajare SS, Hajare SN, Sharma A. Aflatoxin inactivation using aqueous extract of Ajowan (Trachyspermum ammi) seeds. J food sci. 2005;70(1):C29–34.

Lipinski, C.A., Lombardo, F., Dominy, B.W. and Feeney, P.J. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv.

Drug Deliv., 46(1-3):3-26.
Patro, B. K., Kumar, B. R., Goswami, A., Mathur, V. P., & Nongkynrih, B. (2008). Prevalence of dental caries among adults and elderly in an urban resettlement colony of New Delhi. IndianJournal of Dental Research, 19(2), 9
Shankaracharya NB, Nagalakshmi S, Naik JP, Rao LJM. Studies on chemical and technological aspects of ajowan (Trachyspermum ammi (L.) Syn. Carum copticum Hiern) seeds. J Food Sci Technol. 2000;37(3):277–81.
Singh G, Maurya S, Catalan C, De Lampasona MP. Chemical con-stituents, antifungal and antioxidative eff ects of ajwain essential oil and its acetone extract. J Agric Food Chem. 2004;52(11):3292–6.
Umadevi, I., & Daniel, M. (1990). Phenolics of some fruit spices of the Apiaceae.National Academy Science Letters,13(12), 439-441.
Wu, G., Robertson D.H., Brooks C.L. and Vieth, M. 2003. Detailed analysis of grid based molecular docking: A case study of CDOCKER—A CHARMm based MD docking algorithm. J. Compt. Chem., 24(13): 1549-1562.