In-Silico Analysis of Inhibitory Action of Garlic Against Hyperlipidemia By Fas Enzyme

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ABSTRACT:

The volatile antimicrobial substance allicin (diallylthiosulphinate) is produced in garlic when the tissues are damaged and the substrate alliin (S-allyl-l-cysteine sulphoxide) mixes with the enzyme alliin-lyase (E.C.4.4.1.4). Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. It is thought that these properties are the basis of its antimicrobial action. Metabolism of lipid into fatty acids and glycerol and the absorption process in the body involves various kinds of enzymes; one of them is Fatty Acid Synthase (FAS). Excess lipid in the body will cause various diseases, such as obesity and cardiovascular diseases. Treatment for excess in lipid level is usually by using synthetic drugs such as statins, but excessive consumption of drug causes various side effects. Single garlic (Allium sativum) (SG) is widely used as an herb that can treat diverse diseases. SG contains organosulfur compounds including Allicin, Alliin, and Ajoene (E-Ajoene and Z-Ajoene). This study aimed to determine the potential of organosulfur compounds in SG as inhibitors of fatty acid synthase (FAS) enzymes which play a role in the process of lipid metabolism.Based on molecular docking results, it is known that the active compounds found in SG could act as an inhibitor for FAS enzymes which play a role in de novo lipogenesis.

KEY WORDS: ENZYME ACTIVITY, GARLIC, MOLECULAR DOCKING, PHARMACOPHORE

INTRODUCTION

Garlic (*Allium sativum*) is used as a flavouring ingredient in food preparations. Itsthe second most widely used cultivated Allium after onion. It has long been recognized all over the world as a valuable spice for foods and a popular remedy for various ailments and physiological disorders.Garlic grows in temperate and tropical regions all over the world, and many cultivars have been developed to suit different climates.

Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium. It contains a higher concentration of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal effects. Dried, powdered garlic contains approximately 1% alliin (S-allyl cysteine sulfoxide). One of the most biologically active compounds, allicin (diallylthiosulfinate or diallyldisulfide), does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin to allicin.

MATERIALS AND METHODS

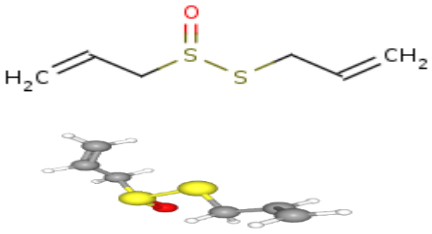
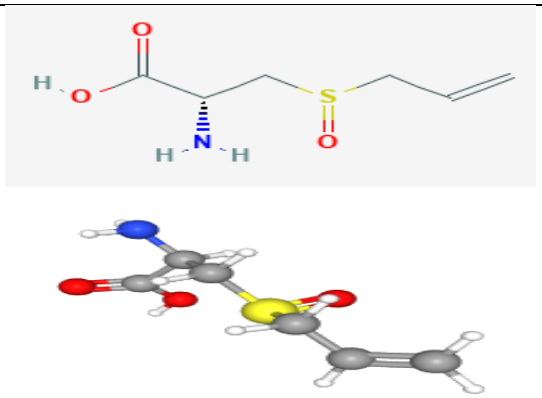
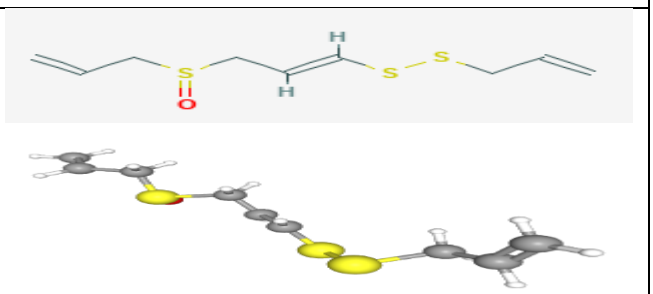
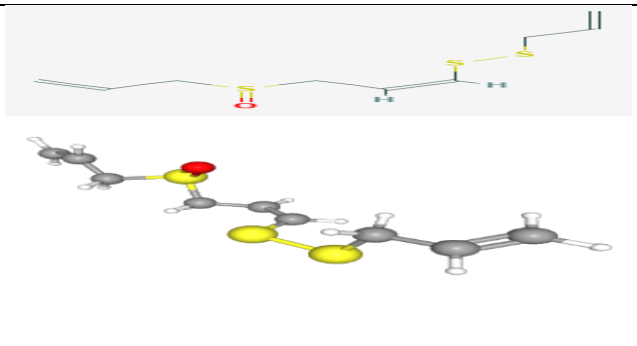
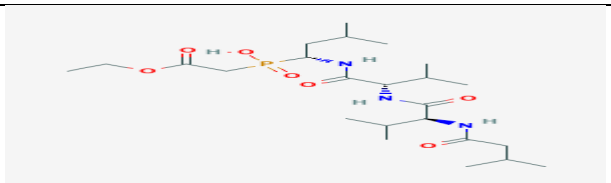
The reported molecular targets responsible for downy mildew of abdiopsis such as salicylic acid,callose, a polysaccharide that is commonly present in these pathogen-induced physical barriers, Multiple independent alleles of dmr1, dmr1-1, dmr1-2, dmr1-3, and dmr1-4, were initially identified. The X-ray crystallised 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. PDB structures include FAS(PDB ID:1DDF) , allinase(PDB ID: 2HOR), Statin(PDB ID: 3LIY), (Z)-Ajoene(PDB ID: 1BWC)etc. 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.

Selection of ligands

The ligands used for docking study were selected from literature. The bioactive compounds that are mainly present in the bulbs of *Allium sativum* were considered for the study. Structures of major compounds present in garlic were retrieved from the PubChem compound database [24] in the SDF file format and followed by conversion in PDB format using the tool Marvin Sketch features an extensive set of functionalities to allow the rapid and precise drawing of chemical compounds, reactions. These structures were used for docking calculation. The reference ligand structure is prepared in prior, using Marvin of ChemAxon by cleaned structure up in two dimension (2D)

configurations. Details of bioactive Compounds considered for the study with their 2D structures were represented in Table 1.

Table 1: List of ligands and their structure

Compound Name	Molecular Weight	Pubchem-Id	Structure
Allicin	162.3g/mol	65036	
Alliin	177.22g/mol	87310	
(E)- Ajoene	234.4g/mol	5386591	
(Z)- Ajoene	234.4g/mol	9881148	
Statin	519.6g/mol	447893	

Molecular docking

It was found that garlic contains allicin (S-Allyl-L-cysteine sulfoxide). Molecular docking is very necessary step performed to study the receptor-ligand interaction to select potential hits in virtual screening which regarded as the basis for structure based drug discovery. Molecular docking was performed in Yet another Scientific Artificial Reality Application (YASARA) an Auto Dock based tool for molecular docking and virtual screening. Y ASARA was used to gain the docking results of the listed compounds with the indicated target proteins. The energy minimized compounds were imported and the docking conformations were performed twice using genetic evolutionary algorithm and the fitness of the docked structures were calculated. The hydrogen bond, Residues, Dissociation Constant, binding energy was calculated using YASARA Software. Here, the vdW term is van der Waal energy. H-bond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively. The output of docking run is sorted based on binding energy. Yasara docking gives positive binding energy. So, more the positive energy indicates the higher likeness among the molecules.

The results of molecular docking showed that statin had the highest binding affinity for FAS enzymes compared to organosulfur compounds (-5.0 kcal/mol). The visualization of binding position using PyMol software showed that the organosulfur compounds (E-Ajoene, Z-Ajoene, and Allicin) had the same binding site with statin in FAS enzyme. The binding site of ligands and protein is shown in Figure 1. Ligands are shown in red (Alliin), magenta (Allicin), blue (E-Ajoene), orange (Z-Ajoene), and white (Statin).

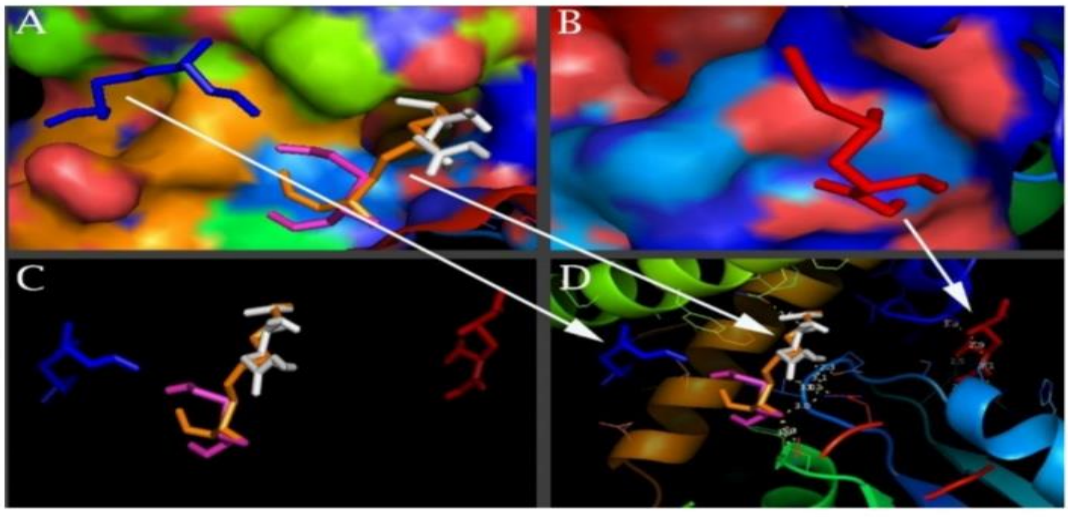


Figure 1.A & B.The visualization of the binding site in organosulfur compounds and statin to FAS enzyme; C.The magnification of the visualization of an organosulfur compound and statin binding site; D.The visualization of all compounds. The white arrow shows the position of organosulfur compounds and statin in the FAS enzyme.

PHARMACOPHORE

A pharmacophore is an abstract description of molecular features that are necessary for molecular recognition of a ligand by a biological macromolecule.Pharmacophore mapping was carried out in the workspace of mole sign module ofVlife MDS 4.3. Dataset of de navolipogenesis was first aligned with reference to most active molecule as template. The most active molecule statin was selected to set it as the reference. The reference molecule is the molecule on which the other molecules of the align dataset get aligned. All spheres in the snapshot indicate all possible pharmacophoriccenters. This pharmacophore model can serve as an effective search filter for virtual screening.

RESULTS AND DISCUSSIONS

The three dimensional structures of the identified target proteins were retrieved from the protein data bank. In order to examine the binding capacity of bioactive compounds in Allium sativum on proteins related to de navolipogenesis, we have used Yasara software to dock the ligand data set to the structure of target protein. Since all the natural ligands(inhibitors) were found to be docked in a variety of conformations and with varying binding energy. From the interaction profile numerous interactions including hydrogen bonding interactions, hydrophobic interaction, Van der Waals interactions, and pi–pi interactions were inspected between selected inhibitors and retrieved Hit molecules with target proteins. Through this methodology of computer aided drug interaction, we examine complexes formed between ligands and interesting targets (often many), for dissimilar types of a particular disease. Target proteins docked with different ligands are shown in Fig 2. The best dock pose was chosen on the basis of high docking score. Top two ligands were selected with better score.

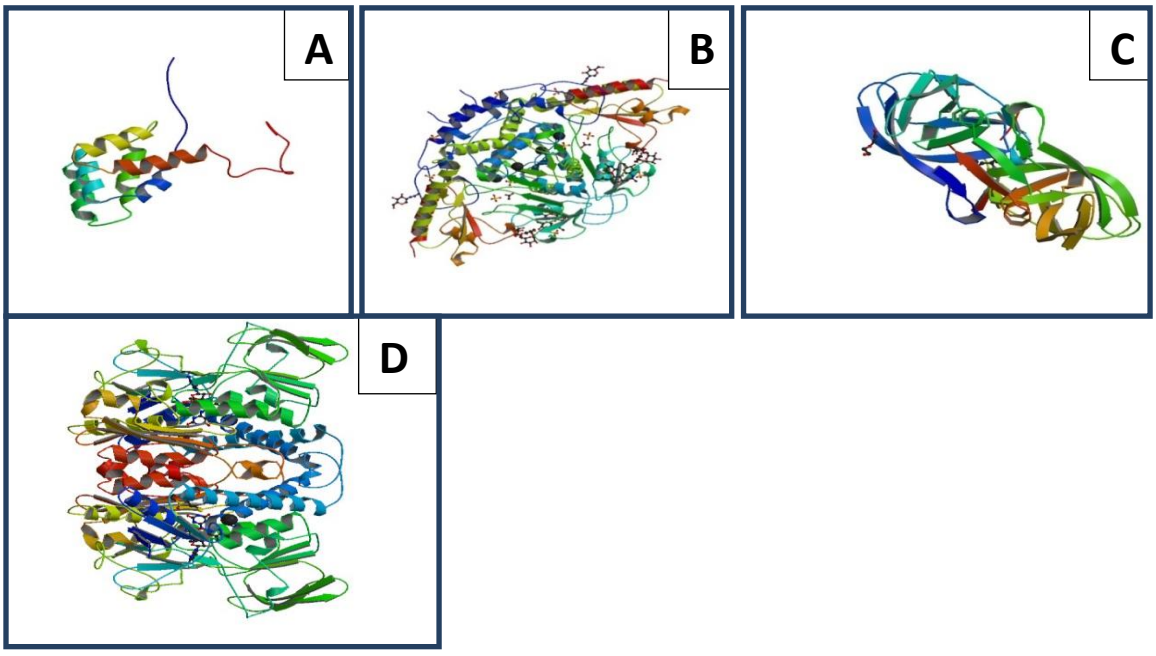
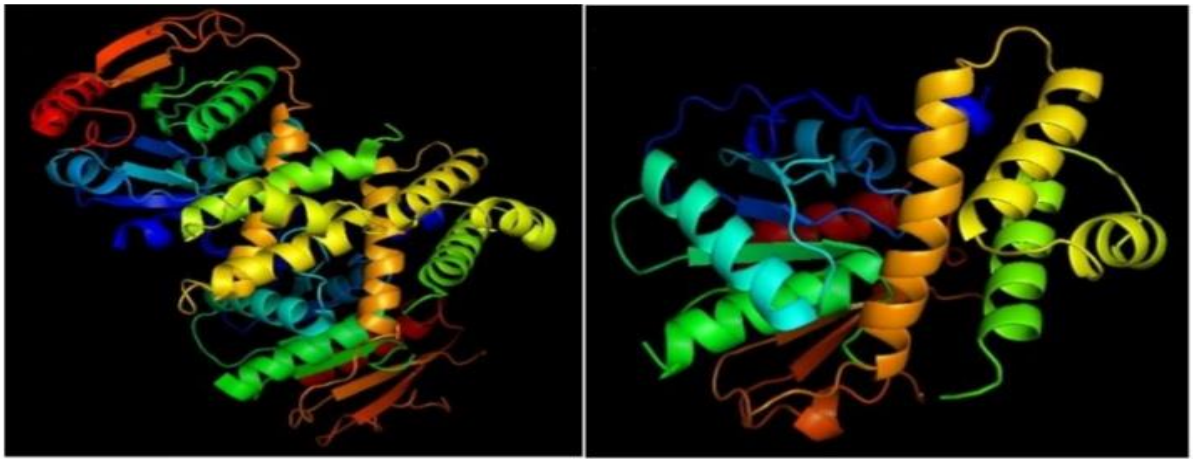


Fig 2: Binding modes and Protein-ligand interaction map of docked complexed ligands with A(FAS), B(Allinase),C(statin), D(Ajoene)



(A) **(B)**

Figure 3. (a) 3D structure of FAS enzyme from RCSB GDP; (b) 3D structure of FAS enzyme after removing water molecules and other ligands.

CONCLUSION:

The results of molecular docking method showed that organosulfur compounds in SG had potential as drug candidates by inhibiting the FAS enzyme. The visualization of the binding site showed that the organosulfur compounds (E-Ajoene, Z-Ajoene, and Allicin) in SG had the same binding site with Statins in FAS enzyme. FAS is an enzyme which plays a role in the biosynthesis of lipid. The inhibition of FAS could reduce the production of fatty acid. Therefore, the SG could be used as an alternative medicine for various diseases caused by hyperlipidemia.

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