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IN SILICO MOLECULAR DOCKING STUDIES OF SOME ISOLATED COMPOUNDS FROM *PISTIA STRATIOTES* FOR A-AMYLASE INHIBITORY ACTIVITY.

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ABSTRACT

The aim of the study to find the mechanism of action of the isolated compounds from *Pistia stratiotes* was explored the α-amylase inhibitory activity by molecular docking analysis used for three phytoconstituents namely beta-sitosterol, daucoterol and sitoindoside I isolated from *P. stratiotes*, to identify whether these compounds interact with the responsible protein (α-amylase enzyme). α wide range of docking score found during molecular docking. Beta-sitosterol, daucoterol and sitoindoside I showed the docking score -3.783, -3.526 and -5.322 respectively. Among all the compounds, sitoindoside I showed best docking score, glide emodel and glide energy and it might be highly considered as safe drug for human. Further *in vivo* investigation need to identify whether isolated compounds from *P. stratiotes* have α-amylase inhibitory activity or not.

KEYWORDS: *Pistia stratiotes* α-amylase, molecular docking, ADME/T properties.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both leading to chronic hyperglycemia. It is often accompanied with

disturbances of carbohydrate, fat and protein metabolism and severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration.[1, 2, 3] WHO projects diabetes to be the 7th leading cause of death afflicting up to 366 million globally with 79.4million individuals being affected by 2030.[4, 5] An effective therapeutic approach for management of diabetes and obesity is to decrease hyperglycemia by retarding and reducing the digestion of ingested carbohydrates. Inhibition of carbohydrate degrading enzymes significantly reduces post prandial increase in blood glucose after a meal by delaying starch hydrolysis. This suppression of post prandial hyperglycemia delays the progression of vascular complications associated with DM. One such enzyme, human pancreatic α-amylase (endo-1, 4-α-D-glucan glucanohydrolase EC 3.2.1.1) plays a pivotal role in DM. It catalyses the initial step in hydrolysis of starch to maltose which is eventually degraded to glucose by α-glucosidases. Alpha amylases are extracellular endo-enzymes that indiscriminately cleave α-1,4 linkages between adjacent glucose units in the linear amylose chain and ultimately generate glucose, maltose, and maltotriose units. This category of industrial enzymes constitutes approximately 25% of the enzyme market. Conversion of starch into sugar syrups such as glucose, maltose, maltotriose, dextrin's sugar, or fructose syrups, etc. are the major part of the starch process trade.[6, 7] The spectrum of amylase application has widened in many different fields, like that clinical, medicinal and analytical chemistry; additionally as their widespread application in starch saccharification and within the textile, food, paper and pharmaceutical industries.[8-12]

Lead discovery is one of the most important processes in rational drug design. To improve the rate of the detection of lead compounds, various technologies such as high-throughput screening and combinatorial chemistry have been introduced into the pharmaceutical industry. However, since these technologies alone may not improve lead productivity, computational screening has become important. A central method for computational screening is molecular docking. This method generally docks many flexible ligands to a rigid protein and predicts the binding affinity for each ligand in a practical time. However, its ability to detect lead compounds is less reliable. In contrast, molecular dynamics simulations can treat both proteins and ligands in a flexible manner, directly estimate the effect of explicit water molecules, and provide more accurate binding affinity, although their computational costs and times are significantly greater than those of molecular docking.[13] Molecular docking predicts the conformation of a protein-ligand complex and calculates the binding affinity. Most docking programs[14-17] involve two operations: "docking" and "scoring." The

first involves the generation of multiple protein-ligand conformations, called "poses," or the sampling of the ligands probable conformations in the binding pocket of the target protein. Most of these programs perform flexible ligand-rigid receptor docking, and some of them are highly capable of predicting poses that resemble the experimental structure for many target proteins.[18]

Pistia stratiotes L., is a free floating, aquatic plant with sessile leaves forming a rosette. The leaves are pale-green, 10-20 cm long and 10 cm wide, spathulate to obovate with a rounded to truncate apex. Around 7-15 veins run parallel from the base. The lower surface is covered with whitish hairs.[2-5] Inflorescence is axillary, solitary, spathulate with a single pistillate flower at base, and 2-8 staminate flowers above. Flowers are unisexual, staminate with two stamens, pistillate with unilocular ovary having numerous[19, 20] ovules, a slender style and penicillate stigma, the fruit with many thin seeds.[21] Its seeds germinate on the hydro-soil and float to the surface within 5 days. Germination can also occur in the dark. *P. stratiotes* does not survive freezing temperatures. Germination does not occur below 20°C. It flowers in summer and give fruits at the end of hot season.[22] The seeds float on the surface for few days, transported by currents and water fowl, before they sink to the bottom. *P. stratiotes* from medicinal point is used as antiseptic, antitubercular and antidysentric. Its extract is used as an anodyne for eyewash and for relieving ear complaints. Its ash is applied to scalp for curing ringworm. Leaf extract is used in eczema, leprosy, ulcers, piles, and syphilis. Leaf extract boiled in coconut oil is applied to the skin in chronic dermatitis.[23] Its concoction is useful for relieving nervous disorders, fever and intestinal bacterial infections. *P. stratiotes* is useful in the treatment of stomach disorder, throat and mouth inflammation.[24]

The aim of the study to find the mechanism of action of the isolated compounds from *Pistia stratiotes* was explored the α-amylase inhibitory activity by molecular docking analysis.

MATERIALS AND METHODS

In silico analysis

Molecular docking analysis of isolated compounds from *Pistia stratiotes*[25]

Preparation of protein structure: The 3D coordinates of crystal structure of α-amylase (PDB: 1PPI) was downloaded from the RCSB protein data bank (http://www.rcsb.org/pdb) set up at Brookhaven National Laboratory in 1971. It is a worldwide repository of information about the 3D structures of large biological molecules, including proteins and

nucleic acids. Water molecules were removed from the protein 1PPI before the instigation of molecular docking. The protein structure was corrected by the utilization of alternate conformations and valence monitor options as some crystallographic disorders as well as some unfilled valance atoms were present in the protein file. The resultant protein file was subjected to energy minimization by applying Chemistry at HARvard Macromolecular Mechanics (CHARMm) force fields. CHARm is a program which provides a large suite of computational tools that encompass numerous conformational and path sampling methods, free energy estimates, molecular minimization, dynamics, and analysis techniques, and mode l- building capabilities (http://www.charmm.org/). After the energy minimization the protein fie was subjected to define and edit binding site option available on tools panel to explore the plausible binding site within the protein (1PPI).

Preparation of ligand: The structures of compounds beta-sitosterol, daucoterol and sitoindoside I were drawn using ChemBioDraw software. ChemBioDraw™ is software from PerkinElmer for development of chemical structures of bioactive compounds. The prepared ligand was then subjected to add the hydrogen bonds and the energy has been minimized using CHARm force field.

Docking analysis: To find out the accurate binding model for the active site of α-amylase enzyme, molecular docking analysis was performed using ligand fit of GLIDE software from Schrodinger (http:/ /www.schrodinger.com /) . Molecular docking analysis was performed using crystal structure of α-amylase (PDB: 1PPI). The structure of crystal structure of α-amylase enzyme (PDB: 1PPI) were obtained from Protein Data Bank (http://www.rcsb.org). The mechanism of ligand position is based on the fitting points. Fitting points are incorporated into the hydrogen bonding groups on the ligand and the proteins. The ligand fit module[26] from GLIDE software was utilized to execute the molecular docking analysis, based on shape-based searching and Monte Carlo methods. At the time of docking, variable trials Monte Carlo conformation was applied where the number of steps depends on the n umber of rotatable bonds present in the compounds/ligands. By default the torsion number is 2, the maximum minimizations steps is 300 and maximum successive failure is 110. During the docking process the top ten conformations were engendered for each o f the compound after the minimization of the energy.[27]

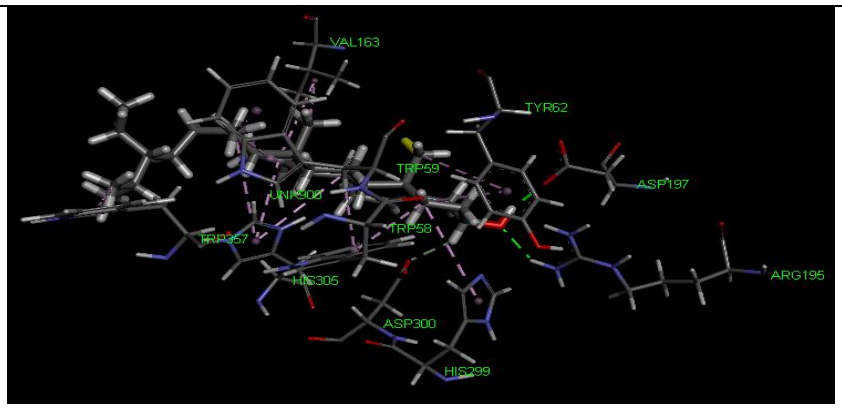
RESULTS AND DISCUSSIONS

In silico analysis

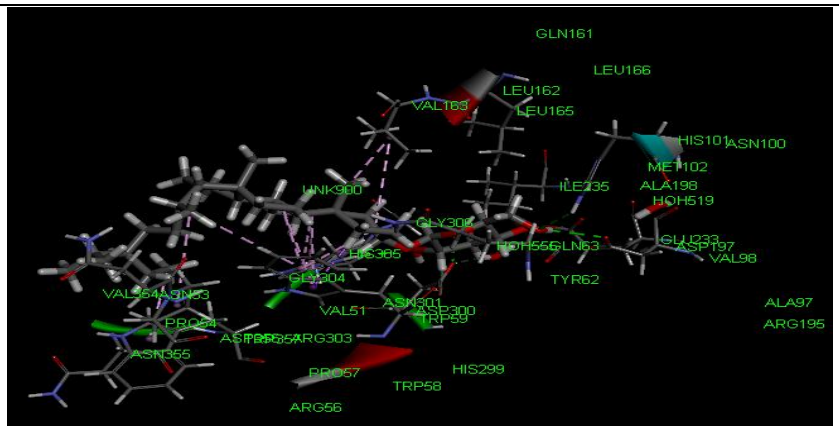
Molecular docking analysis: The crystal structures were refined by removing water molecules and repeating coordinates. Hydrogen atoms were added and charges were assigned to the protein atoms. The docking studies revealed that the van der Waals, electrostatic, and desolvation energies play a key role in binding. In this study, the binding mode of α-amylase enzyme was investigated by doing computational analysis, glide docking. Both glide standard (SP) and extra precision (XP) mode had been introduced, where extra precision mode used for cross validation purpose. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1. Among all the compounds, sitoindoside I showed well docking score, glide emodel and glide energy.

Table 1: Docking results with beta-sitosterol, daucoterol and sitoindoside I in the α-amylase enzyme (PDB: 1PPI).

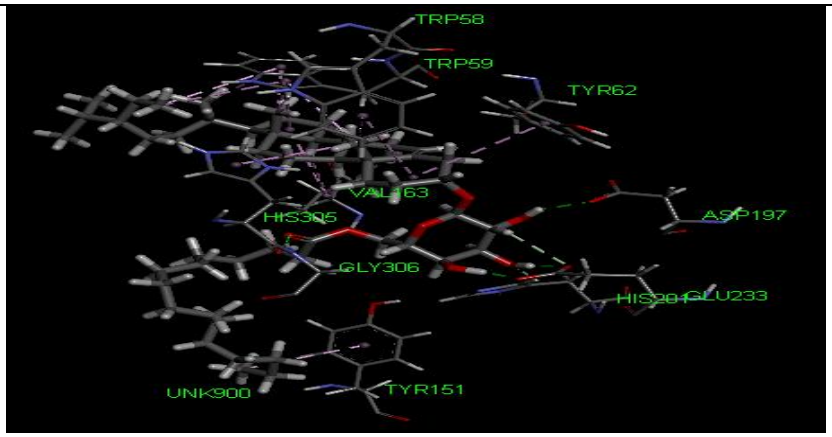
Compound Name	Docking Score	Glide emodel	Glide Energy
beta-sitosterol	-3.783	-37.925	-32.412
daucoterol	-3.526	-24.264	-23.800
sitoindoside I	-5.322	-53.187	-46.638



A. beta-sitosterol



B. daucoterol ….



C. sitoindoside I

Figure 1: Molecular docking analysis of A. beta-sitosterol, B. daucoterol and C. sitoindoside I with α-amylase enzyme (PDB: 1PPI) receptor complex obtained from Glide docking.

Based on the docking studies, the α-amylase inhibitory activity of the selected compounds was found to be decreased in the order of sitoindoside I (-5.322), beta-sitosterol (-3.783) and daucoterol (-3.526). On the basis of the above study, sitoindoside I possess potential α-amylase inhibitory binding site.

CONCLUSION

From the study it was found that, *Pistia stratiotes* could be great source of new α-amylase inhibitor. *In silico* model support that all the isolated compounds from *P. stratiotes* might be α-amylase inhibitor. Among all the compounds, rotundic acid and ursolic aldehyde showed well docking score, glide emodel and glide energy. Further *in vivo* investigation need to identify whether isolated compounds from *P. stratiotes* have α-amylase inhibitory activity or not.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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