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AGONIST STUDY OF PHYTOLIGANDS OF C.IGNEUS TARGETED AGAINST ENZYMES OF ANTIOXIDANT DEFENCE SYSTEM

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ABSTRACT

Inherent defence mechanisms are effective in keeping a variety of pathogens and metabolic irregularities at bay. An analomous behaviour therefore is bound to be scrutinized by the system and dealt with accordingly. In case of oxidative stress, the antioxidant enzymatic system shoulders most of the work. Phytocompounds are gaining unprecedented recognition for their impact in complementary beneficial properties. In this research endeavour, we have performed *insilico* studies to test agonist nature of Phytol, Benzoqinoline and γ – Tocopherol against SOD, CAT and GP_X.

KEYWORDS: Oxidative stress, Phytochemicals, Molecular docking, Antioxidant enzymes.

INTRODUCTION[1][4][16][18]

A physiological imbalance phenomenon called oxidative stress aggregates due to production and accumulation of oxygen reactive species (ROS) in cells and tissues and the inability of a biological system to detoxify these reactive products. ROS dawn many hats (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants) and xenobiotic contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage(oxidative stress) and in turn making the organism susceptible to a plethora of diseases.

Representation-Diseases associated with oxidative stress

Plants store a wide variety of secondary metabolites, which are low molecular weight compounds possessing several biological properties. Plants produce these phytochemicals to protect themselves from oxidative damage; however, recent studies suggest that many of these phytochemicals are able to protect humans from several diseases.

Computer-based receptor-ligand binding as a suitable approach for structure based drug screening and exact phytocompound or combinations of few phytochemicals can be predicted within a few hours by using molecular docking and interaction. On the other hand, the molecular docking tool is used to predict the interaction between a small molecule (ligand) and a macromolecule (protein) that describes the behavioural characterization of small molecules in the binding site of target receptor.

Plant properties[2][3][13][14][15]

Costus igneus is aptly termed a modern-day elixir for its diverse beneficiary properties.

Antioxidant enzymes and their significance[17]

SOD, CAT and GPX constitute the body's primary counter-system to oxidative stress. Levels of these enzymes rise significantly in these altered states. Therefore, they can be employed as biomarkers for referring a system's current state.

Oxidative Stress and phytochemicals[6]

Agonists of SOD, GPx and CAT may increase their activity and thus may overcome stress related ROS induction. Therefore, these three proteins SOD, GPx and CAT have been selected as targets for antioxidant activity of phytochemicals.

MATERIAL AND METHOD

1. Phytochemical Screening[4]

Sample Preparation: The plant was obtained from a residence of Lower Parel, Mumbai. The leaves of *Costus igneus* were collected, washed with distilled water and dried in oven for a week at 50^{o}C. The dried leaves were homogenised using mortar and pestle. Alcoholic extract was prepared. Qualitative screening was then performed.

2. Molecular Docking[5][6][7][8][9][10][11][12]

Proteins selected as targets.

Serial No.	Enzymes (Targets)	PDB ID	Binding Site Coordinates
1	Superoxide dismutase (SOD)	1CB4	X=10.410, Y=87.880, Z=18.620
2	Catalase (CAT)	2CAG	X=58.380, Y=19.080, Z=18.300
3	Glutathione peroxidase (GP_x)	2P31	X=-5.830, Y=03.390, Z=00.200

Phytochemicals selected as ligands.

Serial No.	Phytochemicals (Ligands)	Molecular weight
1	Phytol	296.53
2	Benzoquinoline	179.23
3	γ-Tocopherol	416.68

Protein Preparation

1. Proteins (target) were procured from RCSB PDB in the PDB format file (.PDB).
2. The targets were translated into receptor form by deleting water molecules, inserting polar hydrogens and by giving Kollman charges in Autodock Tools-1.5.6.
3. The grid option was selected and dimensions allocation was done.
4. Save it in the PDBQT format.

Ligand Preparation

1. The phytochemicals were procured from PubChem server in 3D SDF.
2. They were then converted to PDB format using USCF chimera.
3. Save it in the PDBQT format.

Molecular Docking

1. Open the Command Prompt and set the parameters for docking.
2. Input the exhaustiveness as 8 and energy range as 4 for all the studies.
3. Open the log.txt file in Discovery Studio 2016 Client and analyse the results.

The conformer with the lowest binding free energy was used for further analysis.

RESULTS

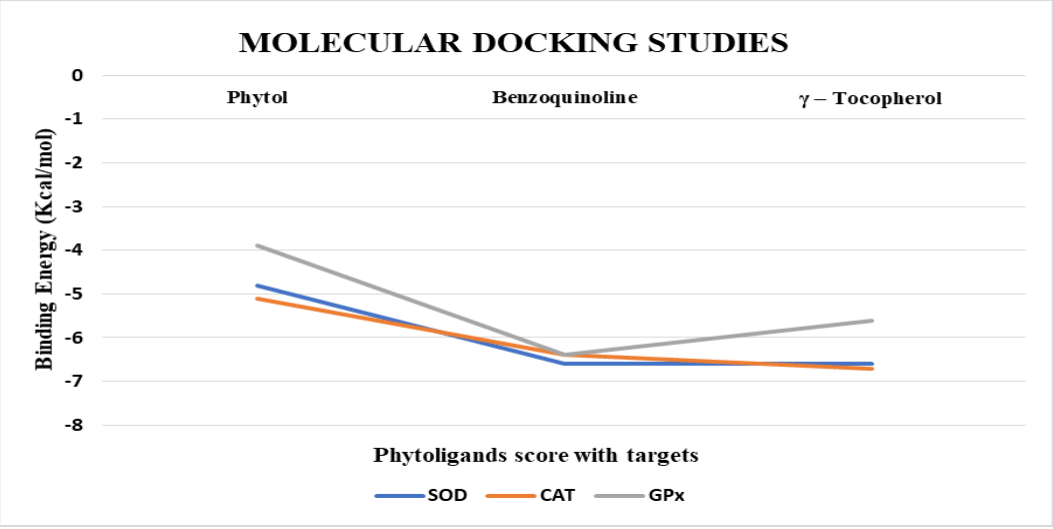
1. Phytochemical Screening.

Qualitative screening shows presence of Carbohydrate, protein, flavonoid, saponin, glycoside tannins and steroids.

2. Molecular Docking.

Table 1: Docking results.

Binding Energy Scores (Kcal/mol)	Phytol	Benzoquinoline	γ – Tocopherol
Superoxide Dismutase	-4.8	-6.6	-6.6
Catalase	-5.1	-6.4	-6.7
Glutathione Peroxidase	-3.9	-6.4	-5.6



Graph 1: Docking studies.

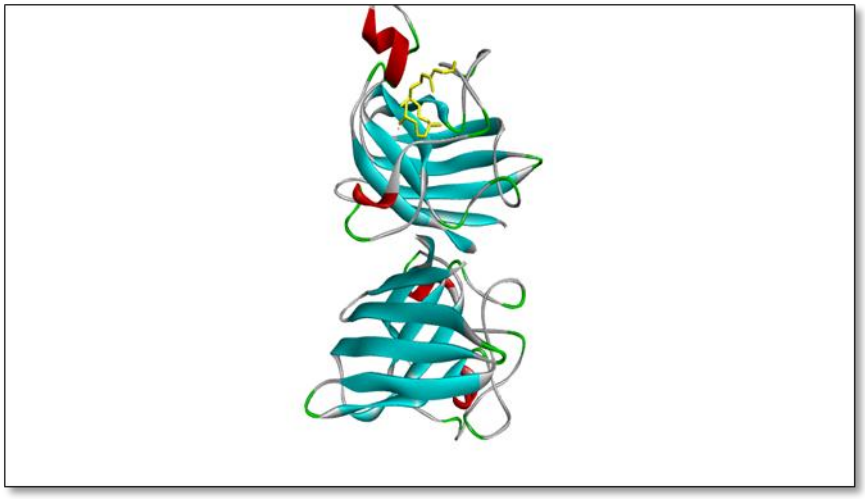


Figure 1: Docking of Superoxide dismutase with Phytol.

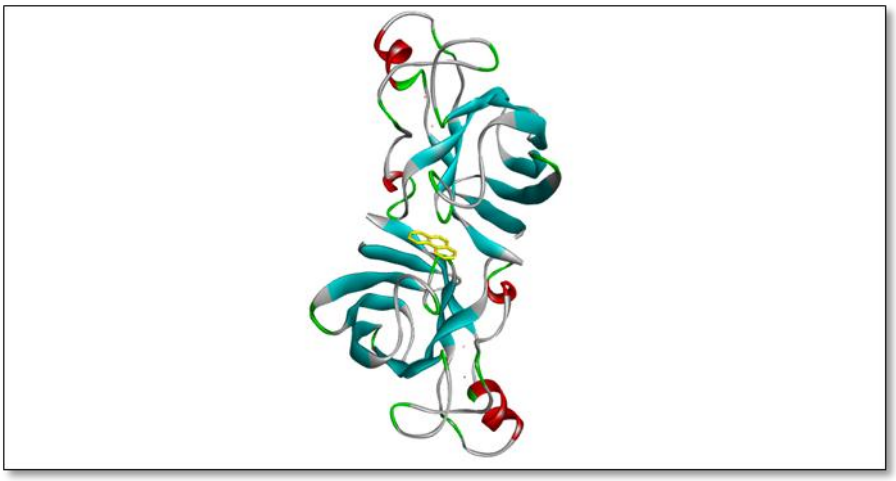


Figure 2: Docking of Superoxide dismutase with Benzoquinoline.

Figure 3: Docking of Superoxide dismutase with γ – Tocopherol.

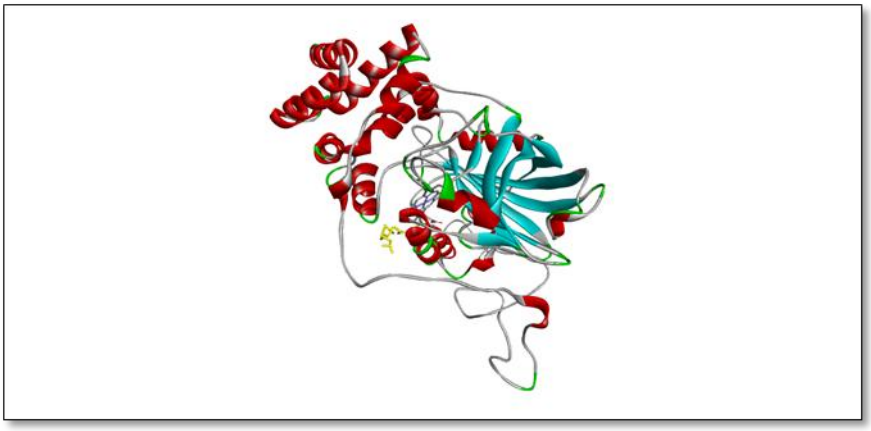


Figure 4: Docking of Catalase with Phytol.

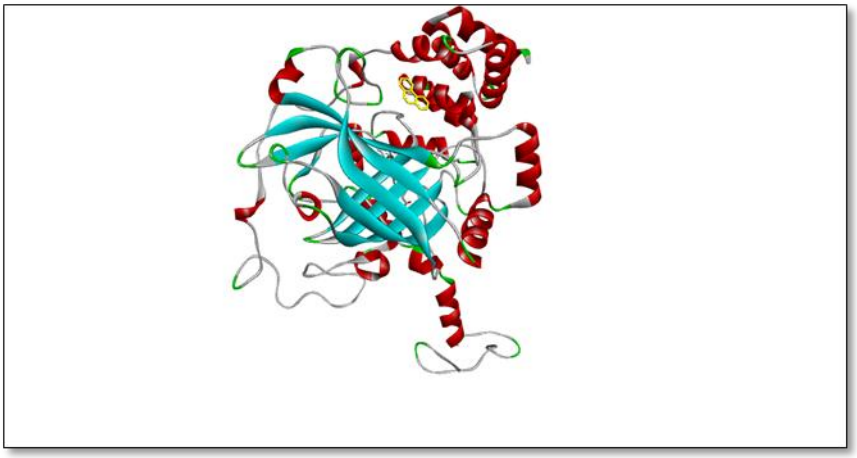


Figure 5: Docking of Catalase with Benzoquinoline.

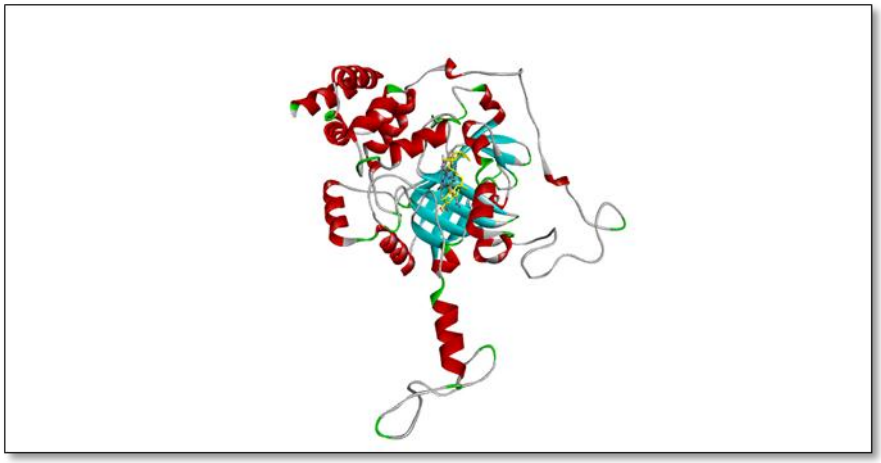


Figure 6: Docking of Catalase with γ – Tocopherol.

Figure 7: Docking of Glutathione peroxidase with Phytol.

Figure 8: Docking of Glutathione peroxidase with Benzoquinoline.

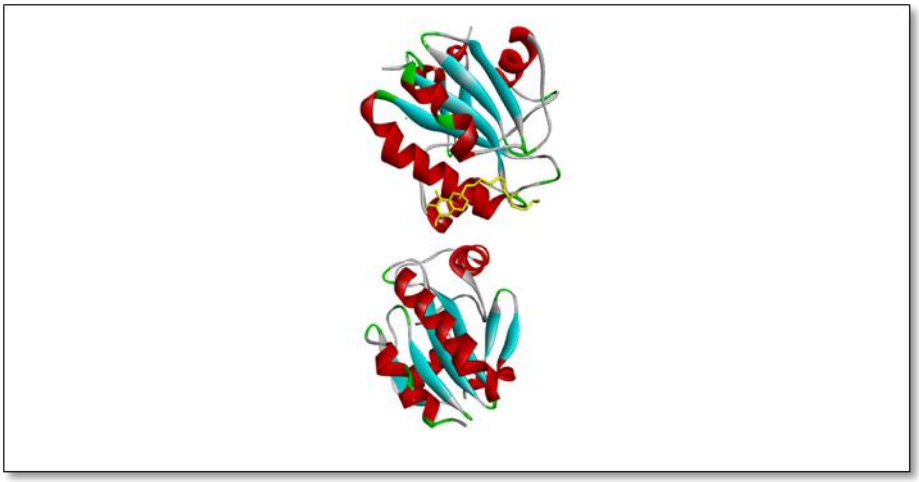


Figure 9: Docking of Glutathione peroxidase with γ – Tocopherol.

DISCUSSION[6][19]

- Molecular docking scores represent energy of interactions when a ligand occupies binding site at certain conformation.
- The conformation of the ligand in a particular pose is three-dimensional arrangement or orientation of its atoms or pharmacophoric groups in which the energy of interactions can be calculated.
- Interaction energy or free energy is the energy required for a ligand can enter into binding pockets and interact with the receptor. The negative sign indicates that the compounds can interact spontaneously with the receptor.
- Negative molecular docking scores usually refer to more attractive interactions over repulsive interactions.
- **Graph 1** depicts that all the three phytoligands have the strongest interaction with catalase enzyme.
- Inference can be made from **Graph 1** that Benzoquinoline is the best candidate for drug (wet lab) studies since it has the least binding energy score with all three of the target proteins.

CONCLUSION[13][14][15]

- Oxidative stress is a potential factor in various disease pathophysiology. By eradicating this malefactor, a safety net for body can be formed devoid of any distress.
- The potential agonist nature of these phytochemicals can be extrapolated to the neuroprotective and antiurolithiatic properties exhibited by the study plant by getting rid of the oxidative stress involved in the aforementioned disease states.
- *Insilico* studies with other phytochemicals should be explored.
- *Invivo* and *Invitro* studies should be carried out to evaluate the stand of this study in physiological systems.

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