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Modeling and Molecular Docking Studies on *Alangium salvifolium* (Alanginaceae) as a Target for Anti-oxidant Enzyme

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Objectives: The present studies pursue at retrieve and draws the active phytocompounds structure of *Alangium salvifolium* and assessing its simulation anti-oxidant enzyme activities.
Methods: Retrieve/draws of the compounds were carried out using *chem.-sketch* software. The 3-D structures of the Phytocompounds were visualized based upon the UV, NMR spectral data along with their energy simulation studies. The antioxidant and enzyme simulation activity were evaluated *in-silico* using the ACD labs,PyRx, RASMOL,PYMOL,Aragslab and Discovery 3.1 studio.
Key Findings: Phytochemicals structure drawing of *A. salvifolium* resulted in the structured and recognition of four phytochemicals. The plant phytochemicals showed significant anti-oxidant enzymes activity enhancer and ROS eliminator through binding to its metal domain receptor.
Conclusion: Phytochemicals were drawing from *A. salvifolium*. To the best of our knowledge, among these phytochemicals, were studied anti-oxidant enzymes metals binding domain to increase the ROS scavenging activity for the foremost time from mimic with molecular docking. Moreover, study of phytochemicals simulation was for the first time from this plant. The plant revealed auspicious increase the antioxidant activities virtual screening. This gives thinking to some of its pharmacological properties and suggests additional antioxidant effects, for as a scavenger as well as anti-oxidant enzyme stimulator, which have not been reported yet.

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Keywords: Alangium salvifolium; phytochemicals; molecular docking study; ROS elimination activity.

1. INTRODUCTION

Plants have been nearly for the therapeutic baseline of various diseases optimistically from the decades by knowledge of Ayurveda in our country. New drug discovery and development Plants are basic and most abundant source of new discoveries for the new drug escort towards various healthcare issues Organic chemical compounds (Phytochemical) found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in relieve of diseases [1]. The effectiveness plant secondary metabolites in the treatment of various diseases may lie in their antioxidant effects [2]. Oxygen is an element compulsory for life; living systems have evolved to survive in the presence of molecular oxygen and most biological systems. Oxidative properties of oxygen play a vital role in diverse biological happening. Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative facts [3].

Alangium salvifolium wang member of the family of Alanginaceae. Ankola and Alangi are its common name in India, and Stone Mango in English. It is a small broad-leaved thorny tree or shrub [4] which is dispersed in tropical and subtropical region such as Bangladesh, India, China Phillipines, Africa, Srilanka and Indochina [5]. A range of ailments including diabetes, jaundice, gastric disorders, protozoal diseases, rheumatic pain, burning sensation, haemorrhages, lung cancer, poisonings, leprosy and many inflammatory patches have been treated by using various parts of the plant [6]. Many bioactive phytochemicals such as assorted flavanoids, phenolic compounds, irridoid glycosides and oxyoglucosides have been isolated by phytochemical screening of it [7]. Previous literature citated that plants indicate the presence of coumarins, triterpenoids and some potent alkaloids in it [8]. Antioxidants enzymes play a very important role in lessening problems related to oxidative stress. The antioxidant enzymes of phytocompounds isolated from appraised medicinal plants Costunolide (20 mg/kg) or Eremanthin (20 mg/kg) for 60 days caused a significant increase in enzymatic activity of SOD, CAT and GPx, when compared with untreated [9,10] Moreover, maximum antioxidant probable, including DPPH radical scavenging (IC_{50}: 11.26±1.29 µg/ml), FRAP (EC_{50}: 26.64±2.17 µg/ml) and TAC (639.55±10.51 mg/g ascorbic acid) was found in the CASR. Donepezil, to prime of our knowledge, the receptor-level mechanism behind this process is no where mentioned. Present study was aimed at the analysis of receptor-level binding affinity of secondary metabolites of *Alangium salvifolium* with SOD, CAT and GPx through molecular docking.

2. METHODS

2.1 Design of Small Molecules (Ligand)

To study inhibition of antioxidant enzyme with sketch small molecules (called as ligand), *Alangium salvifolium* based known phyto-chemicals are selected as listed in Table 1.

2.2 Ligand Preparation

The structures of polycyclic aromatic organic compounds based plant-derived compounds are represented in-silico using Chem-Sketch software [11]. Initially 2-D structures were designed. The 2-D compounds converted to 3-D employing Molecular Mechanics (MM2) method with the help of Chem-Sketch software [12]. The designed molecules are scrutinized for its conformation by ascertaining achievement of global minima. The list of compounds designed along with molecular formula is listed in Table 1, Fig. 2.

2.3 Receptor Enzyme

Electronic structure of AEs is picked as a target protein having PDB reference 2BHH. The protein file obtained from online data base having SOD, CAT, GPx an antioxidant enzymes. The selected enzyme structure was produced by online homology modeling tool in such a way that it has no ambiguities in the form of missing atoms or amino acids. All the heteroatoms (i.e. non-receptor atoms such as water, ions, etc.) were detached followed by assigning Kollmann charges. The Solvation variables were added to the final macromolecule structure using the Addsol utility of Auto-Dock [13]. The place of natural inhibitor in enzyme is served as active site ofselected enzyme and used as it is without any further processing.

2.4 Molecular Docking

Autodock 4.0 [14] is used for docking operations. Initially protein grid was designed using grid design tool of Autodock. Dockings were achieving used both genetic (GA) and non-genetic (Non-GA) algorithm techniques. The genetic algorithm (GA) is the newly adopted conformational search techniques and searches the best possible conformations of ligand inside the active site of enzyme. For each conformational position, it also reports the possible binding energy in the form of ΔG in kcal.mol-1. The selected parameters and settings, which were used for docking, are listed in Table 2. The docking algorithm makes use of force field equations and parameters to calculate the binding energy between ligand and enzyme [15-17]. The binding free energy is the total of van der Waals interactions, H-bond interactions, electrostatic interactions and the internal static energy of the ligand as shown in Equation 1 [18-20].

ΔGbind=ΔGvdw+ΔGhydrophobic+ΔGH-bond+ΔGH-bond(chg)+ΔGdeformation+ΔG

The obtained results of binding energy for Non-GA and GA Dockings for each set of experiments are listed in Table-3. The negative values of docking energies favour the interaction among ligand and enzyme. Though there are chances of non-favourable interactions, the non-favourable results are marked as '*'.

2.5 Receptor Ligands Interaction Study

Docking was ready with the PyRx software (pyrax–www.pyraxviana.com/), in which the result is being obtained on the basis of pose energy. Docking interaction analysis and visualization attempt to place 'Ligand into Binding Sites'. The interaction 2D binding affinity cavity and binding pattern were expressed. The atoms that construct bond between Ligand like inhibitor, and the Binding Site on the protein where the inhibitors bind that Structure were drawn in Discovery studio 3.1 version.

Table 1. Protein (Receptor) general information

S.no	Protein name	PDB ID	Enzyme code	No of chain	Total amino acids
1	SOD	1PM9	EC 1.15.1.1	2	420
2	CAT	1QQW	EC 1.11.1.6	4	2108
3	GPx	1BY	EC 1.11.1.7	1	215

Table 2. General Properties of phytochemicals obtained from *Alangium salvifolium*

S. no.	Properties	Alangium 1	Alangium 2	Alangium 3	Alangium 4
1	Name of chemicals	4(benzoyloxy)methyl -2hydroxyphenoxy tetrahydorxy hexoxone 1,2,3,4,5, pentaium		Tetahydroxy(2hydroxy phenoxy)hexone 1,2,3,4,5 pentaium	Tetahydroxy(2hydroxy phenoxy)hexone 1,2,3,4,5 pentaium
2	Molecular formula	$C_{14}H_{15}O_{14}$	$C_{17}H_{23}O_{12}$	$C_6H_9O_{12}$	$C_{16}H_{12}O_4$
3	molecular weight	407.26	419.36	273.13	268.26
4	Composition				
5	Molar refractivity	81.88	94.46	46.12	76.43

Table 3. Ligand lead energy simulation and pharmacophore specification (Argus lab) results

S.No	Specification	Alangium 1	Alangium 2	Alangium 3	Alangium 4
1	SCF energy	-231.767296889	-300.7076472584	47.3947768704	213.3165388741
2	Geometry	231.819691035	-300.771772957	47.521422858	213.325870339sss

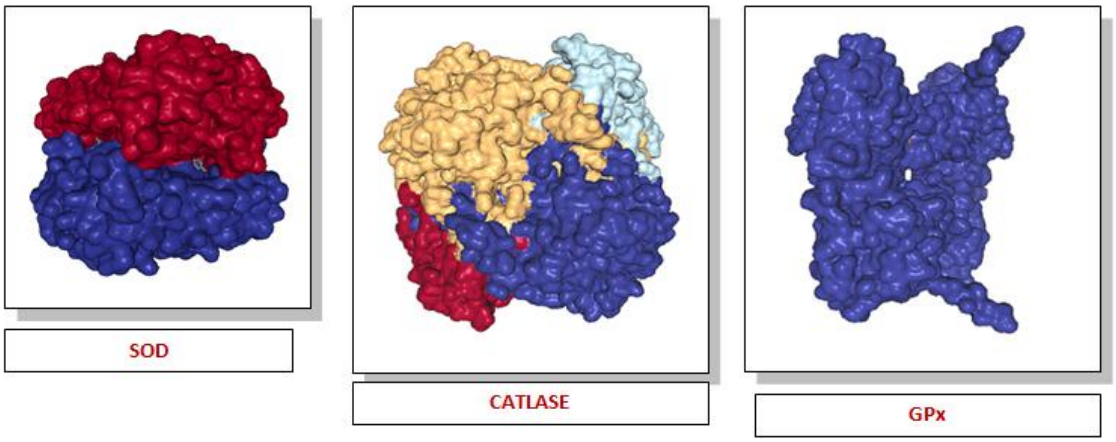

Fig. 1. Antioxidant enzymes model x-ray crystalogrphy structure

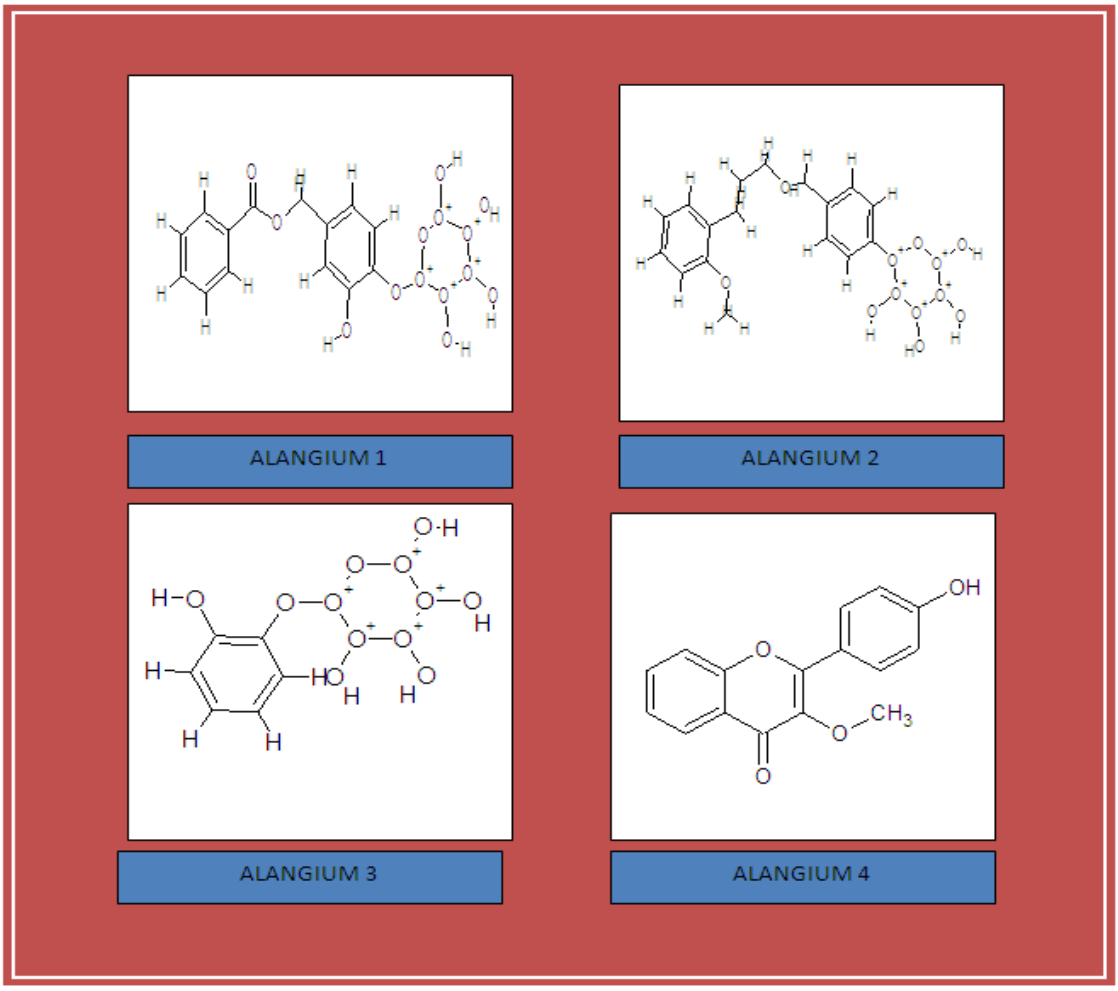

Fig. 2. Chemical structures of Alangium salvifolium Phytochemicals obtained from Chem-Sketch (2D)

Table 4. Mean values of docking energies (kcal/mol) and standard deviation for each skeletal type of *Alangium salvifolium* phytochemicals as liagands with anti-oxidant enzymes enzyme targets

Target	Ligands	Dimension Centre(x=25Ay=25z=25)	No of pose	RSD %lower	RSD %upper	Mean binding
SOD	Alangium 1	X=16.0161,Y=70.1678, Z=15.4010	9	114.74%	57.7%	-7.6
	2		9	52.72%	57.72%	-7.0
	3		9	103.74%	89.09%	-6.3
	4		9	42.62%	39.74%	-7.4
CAT	1	X= 48.844 y= 101.718	9	61.17%	54.60%	-8.9
	2	z=38.2861	9	49.96%	51.85%	-8.3
	3		9	72.23%	67.91%	-6.7
	4		9	47.92%	45.56%	-8.1
GPx	1	X=-12.041 y=22.8816	6	75.32%	78.04%	-7.1
	2	Z=9.2389	4	79.61%	68.17%	-4.7
	3		9	151.01%	122.16%	-5.5
	%4		6	56.07%	61.34	-4.4

3. RESULTS

The SOD, CAT and GPx x-ray crystallography structure proteins were redeemed and examine and it was docked to phytochemical compounds *from A. salvifolium*. The results are presented as follow:

3.1 Retrieving Three Dimensional Structures of Anti-oxidant Enzymes

The structure of antioxidant enzymes (AE) SOD, CAT, and GPx with PDB Id: 1PM9, 1QQW, 1BY were as taken for further analysis. This structure was scaned to know details of the AE molecule. The secondary structure information about AEs proteins have been retrieved from PDB sum database. The topology of the different secondary structures of AEs and the amino acid residues in which each helices and sheets are established. The three-dimensiona structures of AE are composed of similar α/β TIM barrels. The symmetry of the TIM barrel is disrupted by the presence of two short anti-parallel β-strands at the N-terminus connected by a tight turn closing the bottom of the barrel [21]. The PDB file was downloaded and viewed in Ras Mol and their various models are given in Table 1 Fig.1a, b,c.

3.2 Binding Site Prediction – Q-Site Finder

The protein structure of AE was given as load to Q-Site Finder tool and binding site of the protein was prophesy. Ten best 'binding sites' were predicted. The amino acids and the atoms involved in the site were listed.

3.3 Drawing Three Dimensional Structures of Inhibitors

3.3.1 Chemsketch (ACD labs)

The chemical structure of the patronaging *A. salvifolium* APIs collected from literatures were drawn in ChemSketch and visualise into 2D and 3D dimensional structures and its general properties were summarized in Table 2 Fig. 2.

3.4 Making legends Pharmacophore by Using of ArgusLab

Ligands energy extent and its simulation properties were assess by using of Argus Lab software. Various energy level calculation and visualization was summarized in Table -3 and Fig. 3.

3.5 Docking (Autodock-Viana)

Using PyRx (Autodock 4-0) version, the receptor, AEs.pdb file and the ligand pdb file were taken and the protein side chain molecules were detached with the help of various tool controls for their perfect visualization. Hetero atoms were removed and the molecule was used for docking. The binding site molecules were kept as separate PDB file and that was used for the analysis. Then, the protein file and the ligand pdb file were loaded and docking studies were performed. The best docked conformation with its binding energy was found and details are given Table 3. While executing docking the protein and ligand appeared in a grid as shown below and the various binding configurations are

analyzed and finally the list of number poses are given as output and saved as .SDF files. Hydrogen bonds were appended and energy was minimized using CHARMm force field. Further, docking studies also carried out using Discovery studio 3.1 version.

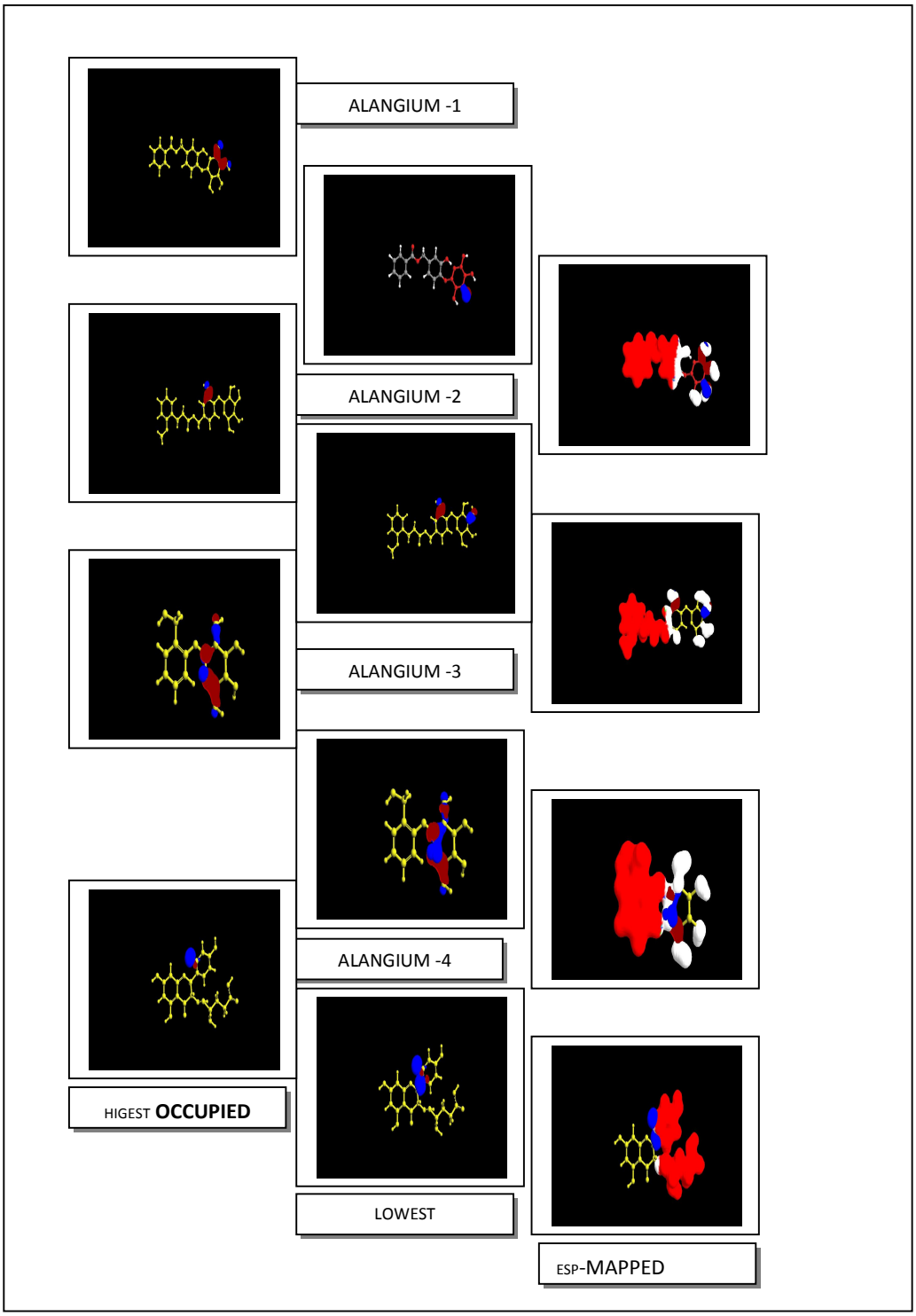

Fig. 3. Ligand leads acquired energy simulation images produced by Argus lab software

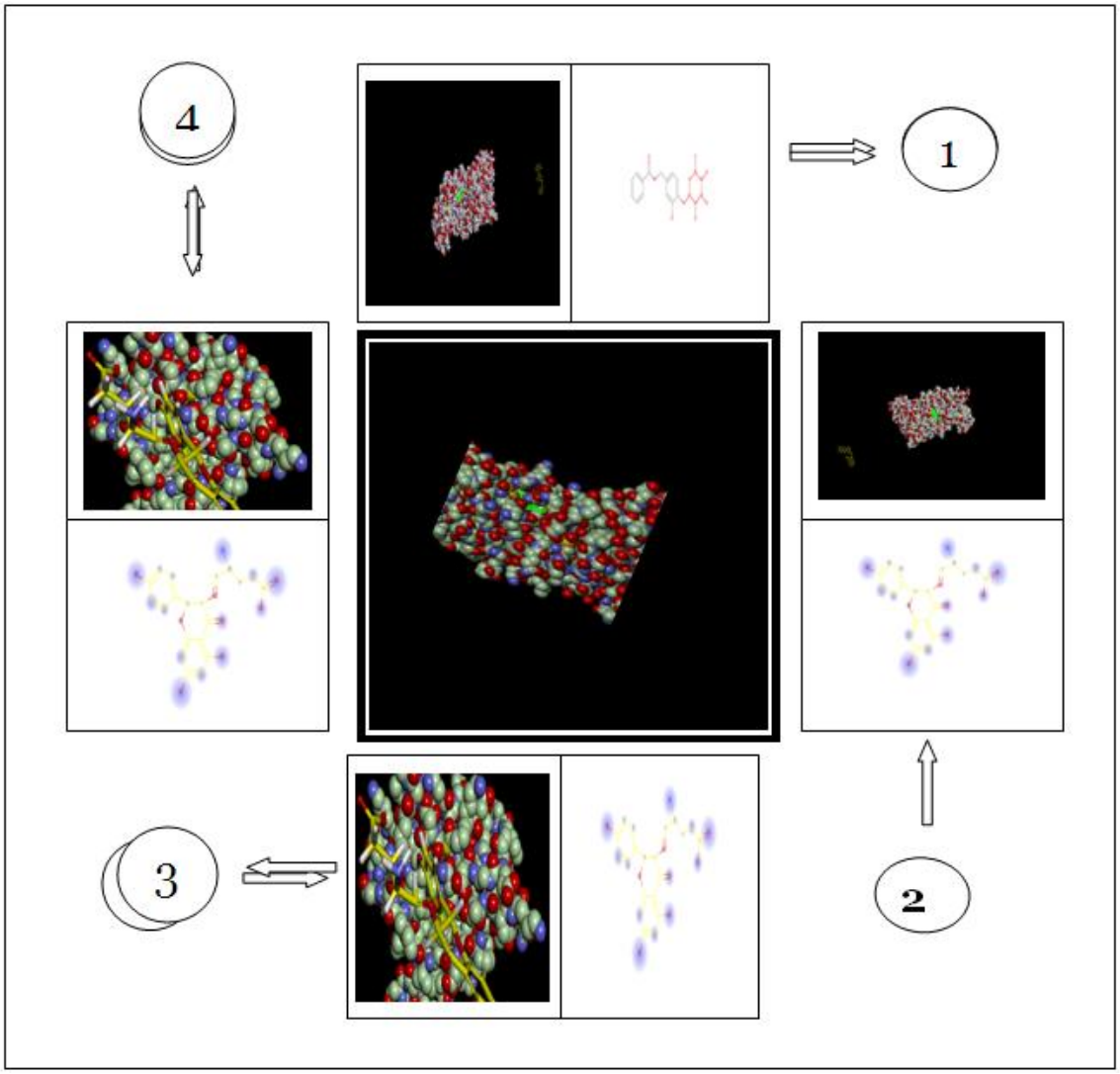

Fig. 4. The binding analysis and the ligand interaction between SOD (1PM9) and phytocompound of *A. salvifolium*

The *A. salvifolium* phytochemicals effectively docked in to the binding site of AEs protein indicating that they are efficient drug compounds. All these binding ligands viz., 4(benzoyloxy) methyl-2hydroxyphenoxy tetrahydorxy hexoxone 1,2,3,4,5, pentaium Tetahydroxy(2hydroxy phenoxy)hexone 1,2,3,4,5 pentaium, Tetahydroxy(2hydroxy phenoxy)hexone 1,2,3,4,5 pentaium showed efficient docking as indicated by binding energy and all are efficient inhibitors.

3.6 Interaction Analysis

Interaction between receptor (Protein) and ligands (Phytocompounds) on the basis of ligands pharamcophore belonged and receptor protein binding cavity. Inside the cavity ligands was oriented in different pose and making a weak hydrogen or hydrophobic bond formation. Receptor protein amino acid and ligands possible potential arms interact each other and make a binding affinity the interaction of protein and Phytocompounds of *Alangium salvifolium* were curtail and depicted in 2D and 3D structure in Figs. 4,5,6.

4. DISCUSSION

Antioxidant enzymes have ability to stabilizing, or deactivating free radicals before they attack

cellular components. They play key role by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. For the past decade, countless studies have been devoted to the beneficial effects of antioxidant enzymes [22]. The SOD enzyme demolishs the superoxide radical; however, as a result of that it creates hydrogen peroxide, which also has high toxic properties [23]. It has been reported as one of the most important antioxidant defense enzyme that scavenge superoxide anion by converting to hydrogen peroxide thus diminish the toxic effect caused by this radical [24]. Catalase is a tetrahedrical protein, constituted by four heme groups which catalyze the dismutation of hydrogen peroxide in water and oxygen [25] Phenol oxidases are copper proteins catalyse the aerobic oxidation of certain phenolic compounds to quinones. Polyphenol oxidase is one of the major enzymes that have a role in the biosynthesis of lignin and defense against water stress by scavenges H_2O_2 in chloroplasts [26]. Glutathione S-transferases (GSTs), a family of cytosolic multifunctional enzymes. It catalyzes the conjugation of glutathione with a variety of reactive electrophilic compounds, thereby neutralizing their active electrophilic sites and subsequently making the parent compound more water soluble. Glutathione peroxidases are substantially more efficient on a molar basis than other enzymes [27]. Glutathione peroxidase acts as a radical scavenger, membrane stabilizer and precursor of heavy metal binding peptides.

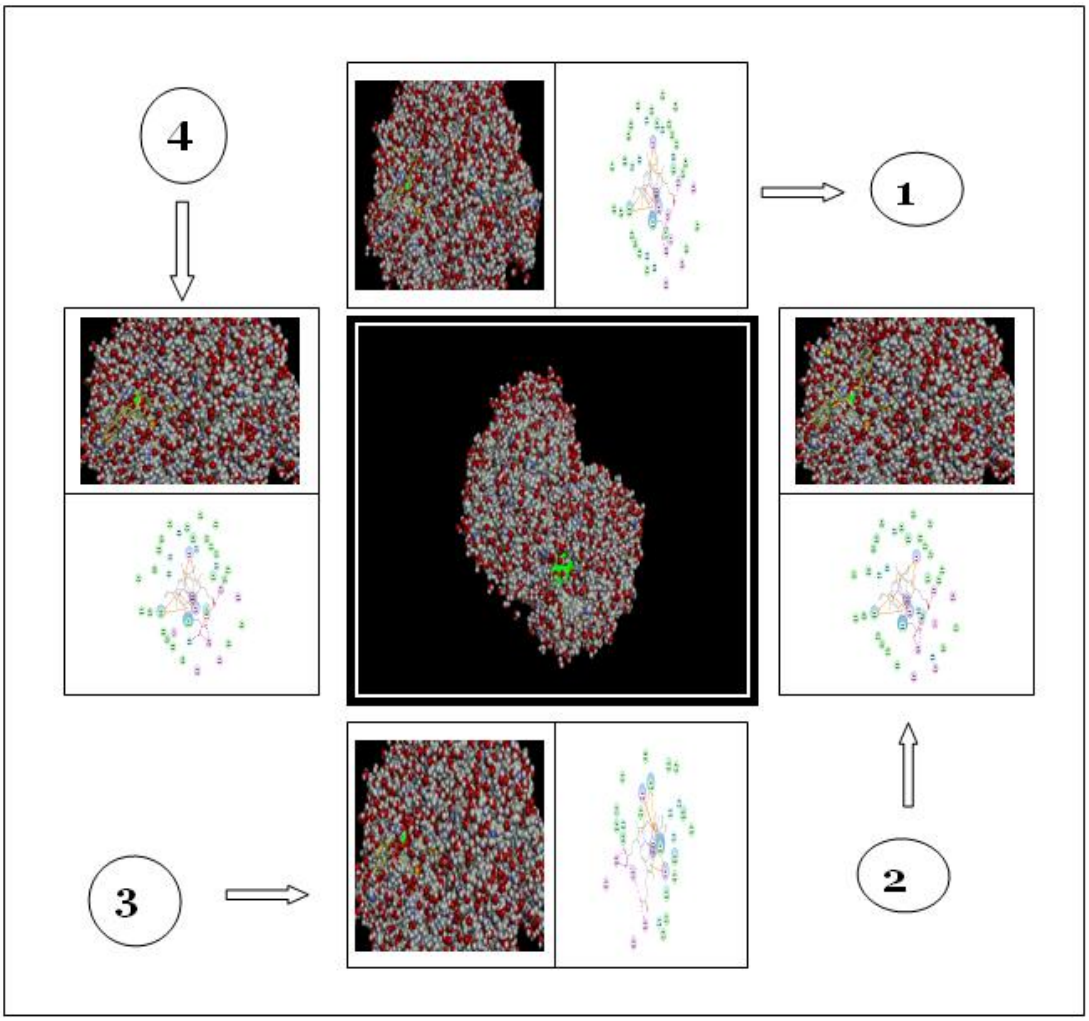

Fig. 5. The binding analysis and the ligand interaction between Catalase (1QQW) and phytocompound of *A. salvifolium*

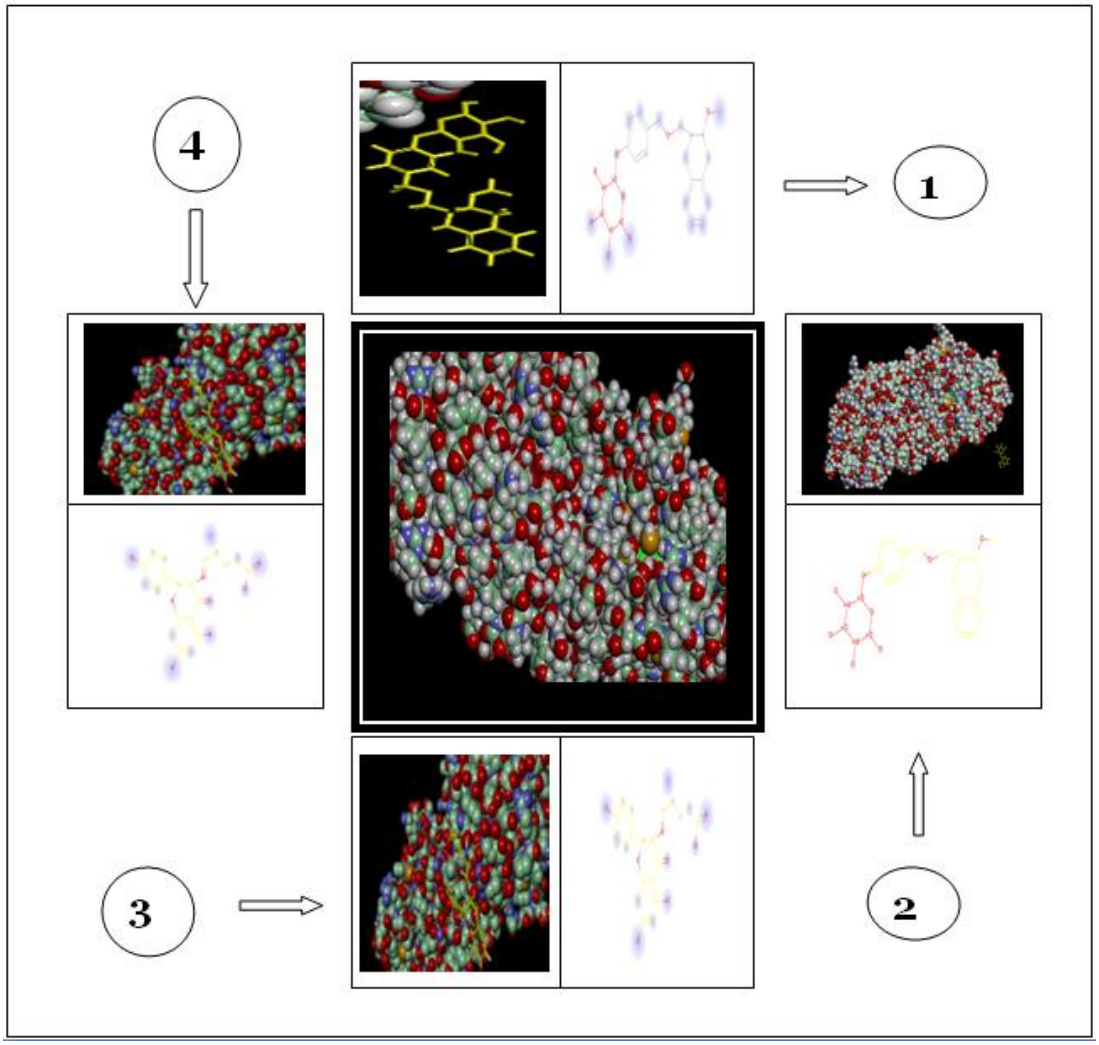

Fig. 6. The binding analysis and the ligand interaction between GPx (1BY) and phytocompound of *A. salvifolium*

NOS enzyme crystal structure complexed with inhibitor was taken for our study to discover novel hit molecule for antioxidant drug discovery. The reference ligand was docked into the active site of the enzyme. The amino group of reference ligand was found to interact with positively charged amino acid Glu592 and non-polar amino acid Trp587. The phyto-compounds selected for A. salvifolium in this study was made to dock into the active site pocket of the antioxidant enzymes(SOD) and found that the compound code Alangium-1(4(benzoyloxy)methyl-2hydroxyphenoxy tetrahydorxy hexoxone 1,2,3,4,5, pentaium) was found to be best docking score as an binding affinity (-kcal/mol. The closer analysis of the compound was analyzed and found that the compound was found to interact with the amino acid Ser457, Thr231 and the benzyl group is stacked with the non-polar amino acid Trp409. The 3-dimensional view of this molecule reveals that the compound was well fitted into the active site cavity which made this molecule more effective binding than the reference ligand. Furthermore, the nitro group and methoxyphenyl group was well surrounded by the non-polar amino acids. The binding analysis and the ligand interaction 2D, 3D diagram was depicted in the Fig. 4.

The target receptor of catalase(CAT) showed the excellent docking score to liagand (Alangium-1) further discuss about this compound binding analysis and interactions, the amino acid Ser257,Thr245 and Ala 235 donates

one hydrogen atom to the compound and the chloro benzilic group was found to be firm interact with two stacking interaction with non-polar amino acids Trp409 and Phe584. Furthermore, the compound is fully surrounded by the non-polar amino acids such as Val167, Ala266, and Ile324 which made this compound possess better docking score than others. The binding analysis and the docking score of the compound were depicted in Fig. 5.

The GPx antioxidant enzyme showed the good docking score against the Alangium-1. Further, the structure-activity relationship of this compound reveals that the metal binding domain group is showing a stacking interaction with Trp209. Due to the presence of bulky heme group present site on the both the side of this compound, the compound tends to powerful its activity on binding with the enzyme [12]. The binding analysis and the ligand interaction of the compound were depicted in Fig. 6.which made this compound more active than without these phytocompounds because the Alangium phytocompounds firmly bind with metal binding domain which give superior stability to bind the metal group for anti-xoidents enzymes. Therefore increase its scavenging activity for reactive oxygen species (ROS) and reduce the oxidative stress. Inside the cell [13]. The binding analysis and the ligand interaction of the GPx and Alangium-1 phytocompound were depicted in Fig. 6. The antioxidant enzymes studied in this research, the 3-dimensional representation of this interaction reveals that the interaction between receptor and ligands is closed from the metal binding domain site.

5. CONCLUSION

All the four components have more or less similar docking energies and so all the four compounds can be used for good binding affinity in different pose site AEs activity. It might be expected that the active components isolated from *A. salvifolium* phytochemicals would have some pharmacological actions to promote the oxidative radical scavenging activity SOD, CAT and GPx enzymes. Further this may be confirmed by drug trials in *In-vitro* and *In-vivo* models to find out the optimum dose and its efficiency in binding actively AEs.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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