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Research Article

Molecular Modeling and Docking Analysis Of *Callosobruchus maculates* Cytochrome B Proteins with Bio Active Compound Phytolfrom *Glinus lotoides* and Synthetic Pesticide Pirimiphos-Methyl

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ABSTRACT
Cytochrome b is a component of respiratory chain complex III, also known as bc1 complex or ubiquinol-cytochrome c reductase. These complexes are involved in electron transport and generation of ATP and thus play a vital role in electron transport chain. Phytol is a phytochemical derived from ethyl acetate extract of *Glinus lotoides.* Cytochrome b modeled and the structure was used for *In-silico* binding efficiency and comparative analysis of cytochrome b with Phytol and synthetic pesticide were showed good docking score and binding affinity when compared to control. This study will be used in broad screening of the protein in the respiratory process and can be further implemented in designing pest control.

Keywords: Homology modeling, Cytochrome b, Ramachandran plot, Docking.

INTRODUCTION

The cowpea weevil, *Callosobruchus maculatus* (Fabricius) is a major pest of cowpea. Cowpea, one of the main sources of protein for teaming populaces in poor tropical countries, is usually prone to attack by *C. maculatus*. The infestation usually commences from the field where the crops are grown and continues during storage. However, the problems associated with insecticides for its safe, insect resistance, health hazards and cost, have led to continuous expedition by food protectionists. Phytochemicals are natural substances particularly have a low toxicity to non-targeted organism[1] and degrade rapidly in the environment[2,3] than the synthetic insecticides.

Glinuslotoides L. (Molluginaceae) is an annual or short-living perennial prostrate herb[4] the seeds of which are traditionally used in Ethiopia as anthelminthics[5,6] and in India and Pakistan as antifungal and antitumor[7,8]. The taenicidal activities against *Tenia saginata* and *Hymenolepisnana* worms[9-11], and the antitumor activities against Dalton's ascitic lymphoma in Swiss albino mice[8] have been evaluated. The pharmacological activities of the plant have been attributed to its saponins and flavonoids[5,6,8–11]. Phytochemical investigation of *G. lotoides* afforded several hopanetriterpenoidalsaponins (glinusides A-I; lotoideside A-F and succulentoside B), flavonoids (vicenin-2; vitexin-2"-O-glucoside; apigenin-7-O-glucoside, isovitexin, and luteolin-7-O-glucoside) and isoflavonoids[12–16].

Cytochrome b is a component of respiratory chain complex III, also known as bcl complex or ubiquinol-cytochrome c reductase. These complexes are involved in electron transport and generation of ATP and thus play a vital role in the cell. Cytochrome b is a membrane bound enzyme that catalyses the transfer of electron from ubiquinol to cytochrome c, coupling this process to the translocation of protons across the inner mitochondrial membrane. In eukaryotic this enzyme complex is composed of 11 polypeptide subunits. Cytochrome b is one of the subunit encoded by the mitochondrial genome. This cytochrome b is fundamental for the assembly and function of the complex III, together with cytochrome c1 and iron sulfur protein (ISP). Cytochrome b is capable for the oxidation and reduction reaction in the electron transport chain. Regulation of this gene results in damage in electron transport chain[17]. In the present study we have targeted this protein with the phytochemical Phytol from *Glinus lotoides* and synthetic pesticide.

MATERIALS AND MATERIALS

Sequence Alignment

The FASTA sequence of cytochrome b (Accession number A4ZVR8) from *Callosobruchus maculatus* was retrieved from the UniProt database. Comparative modeling usually starts by searching the PDB of known protein structures using the target sequence as the query[18]. This search is generally done by comparing the target sequence with the sequence of each of the structure in the database. The target sequence was searched for similar sequence using BLAST (Basic Local Alignment Search Tool)[19] against Protein Database (PDB). The BLAST results yielded X-ray structure of 1BCC (Cytochrome Bc1 complex from Chicken)[20] with 71% identity to our target protein.

Homology Modeling

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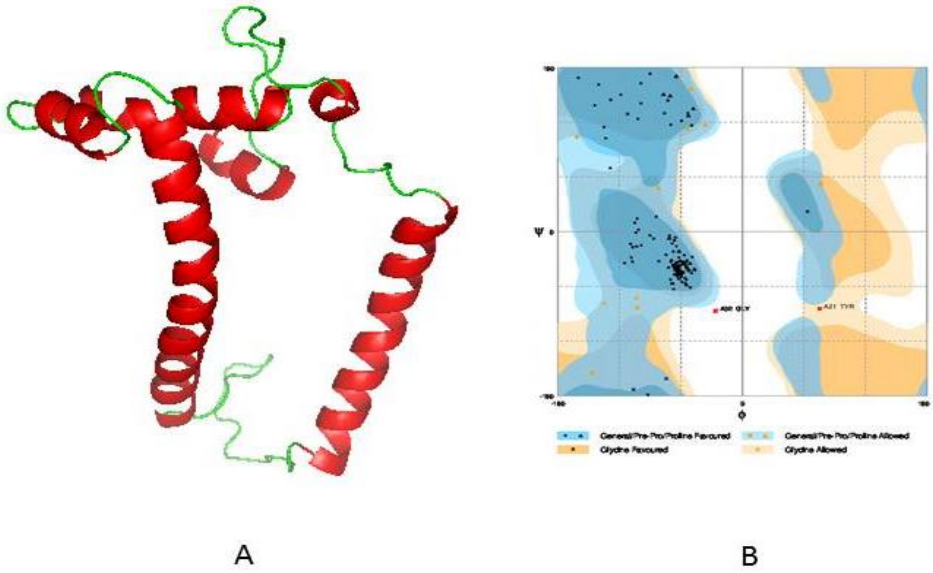

A B

Figure 1: Results from structural studies on cytochrome b. A) Three dimensional structure of the modeled protein. B) Ramachandran plot of the predicted structure.

Table 1: GC- MS analysis- percent peak area of ethyl acetate extract of *Glinus lotoides.*

Peak No.	Name of phytochemicals	R. Time	Area %
1	1,2,3-Propanetriol monoacetate	6.159	4.72
2	1-Acetoxy-2,3-dihydroxypropane	8.504	6.72
3	LOLIOLIDE	15.346	1.60
4	2,6,10-trimethyl,14-ethylene-14-pentadecne	15.744	4.62
5	3,7,11,15-Tetramethyl-1-hexadecanol	15.999	0.88
6	(2E)-3,7,11,15-tetramethylhexadec-2-en-1-ol	16.190	1.40
7	Phytol	18.480	17.43
8	NEOPHYTADIENE	19.377	3.19
9	1,2-Benzenedicarboxylic acid, mono[2-ethylhexyl]ester	22.060	3.52
10	Hexacosane	22.488	1.04
11	Hexatriacontane	23.231	2.29
12	n-Hexatriacontane	23.944	2.08
13	Tetratriacontane	24.637	3.69
14	N-Tetratetracontane	25.305	2.68
15	Alpha-Tocopherol-beta-d-mannoside	25.756	1.61
16	Tetracosane	25.963	9.63
17	gamma-Tocopherol	26.272	4.42
18	Tetratriacontane	26.636	2.42
19	(3β)-Ergost-5-en-3-ol	27.110	3.00
20	Stigmasterol	27.316	4.84
21	n-Hexatriacontane	27.417	3.62
22	(3beta,24S)-stigmast-5-en-3-ol	27.881	11.40
23	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	30.055	3.20

The amino acid sequence of cytochrome b was retrieved from UniProtKB in FASTA format. The template protein for carrying out the homology modeling of cytochrome b was selected based on the BLAST result of the sequence. The 3D structure of template protein for cytochrome b was retrieved from PDB with the PDB ID 1BCC. The homology models of cytochrome b were predicted using MODELLER v9.12[21] and the best model was chosen based on DOPE (Discrete Optimized Protein Energy) score. The stereo chemical quality of the predicted best model was validated using Rampage[22].

Binding site Prediction

The active ligand binding site or the binding pocket residues were predicted using online CASTp[23] server. It uses the pre calculated PDB surface features such as void volume and accessible surface of the pocket. CASTp server was utilized to identify the active site of the modeled protein. CASTp server was employed to study the probable residues in active site region[24]. Further, docking studies were initiated using AutoDock 4.2.6.

Preparation of ligand molecule

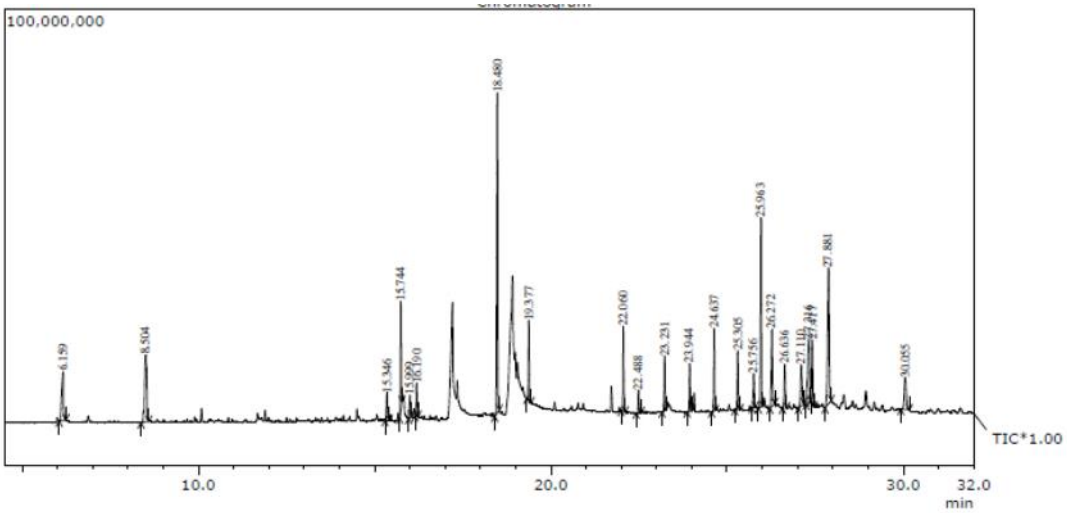

Figure 2: GC- MS chromatogram of ethyl acetate leaf extract of *Glinus lotoides.*

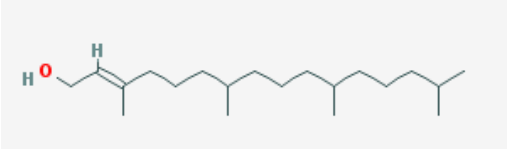

Figure 3: Chemical structure of phytol.

Table 2: Inhibitory activity of Phytol isomer on *Callosobruchus maculatus* Cytochrome b as a target protein.

	Phytol	Pirimiphos-methyl
Binding amino acid Residues	PHE`142/HN, TRP`138/O	HIS`48/HD1
Binding Energy (kcal/mol)	-3.15	-2.01
Inhibition Constant uM	4.87	33.83
VDW energy	-7.23	-4.06
Ligand efficiency	0.15	0.11
Electrostatic energy	-0.1	-0.04
Torsional energy	4.18	2.09
Unbound energy	-1.08	-0.82
refRMS	145.9	154.25

GC-MS analysis of *Glinus lotoides* ethyl acetate extract showed several phytochemicals where Phytol shows maximum area. Phytol was already reported for its antimicrobial activity by damaging cell membrane which leaks potassium ions[25]. The structure of ligand molecule was retrieved from Pubchem as 3D SDF format and converted into PDB format using Open Babel software[26].

Docking Studies

The docking analyses of the bioactive compound obtained from GC-MS were carried out by means of the Autodock tools (ADT) v1.5.6 and Autodock v4.2 program, from the Scripps Research Institute. To run autodock, we used a searching grid extended over the selected target proteins; polar hydrogens were added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger type were assigned and the nonpolar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set. Phytol was docked to the target protein with the molecule considered as a rigid body and the ligands being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 E. The search was carried out with the Lamarckian Genetic Algorithm[27]; populations of 150 individuals with a mutation rate of 0.02 have been evolved for 10 generations. Evaluation of the results was done based on comparison of the predicted binding energy of phytol with the control.

RESULT AND DISCUSSION

Structure prediction

For the present research investigation we retrieved the amino acid sequence of cytochrome b *(Callosobruchus maculates)* from Uniprot. By using our sequence cytochrome b (Accession number A4ZVR8) of *C. maculates* as a query sequence, the three dimensional model of cytochrome b was generated by homology modeling. The modeling of the three dimensional structure of the protein was performed using modeller with Cytochrome Bc1 complex from Chicken as template. The template showed 71% identity with the target. The E-value for the chosen template was 7e-73.The three dimensional structure of the target protein (Fig.1.A) obtained after homology modeling was validated further.

The stereo chemical quality of the predicted model was evaluated after the refinement process using

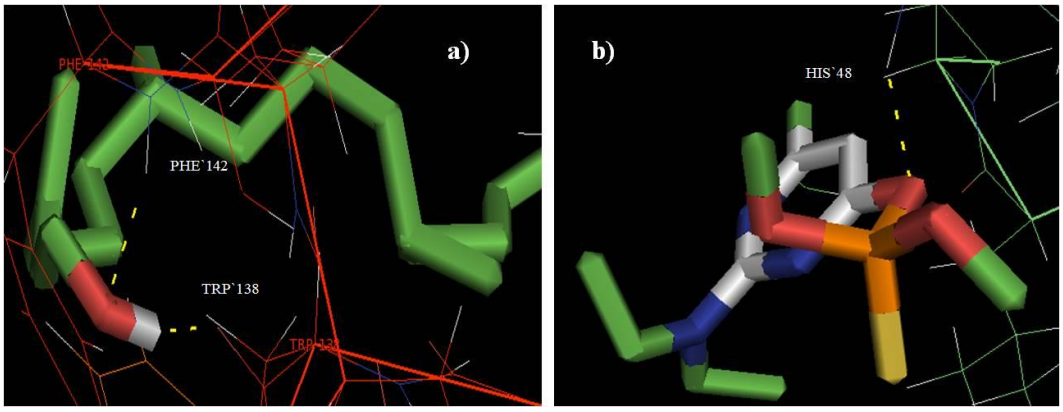

Figure 4: Docked orientation of a) Phytol b) Primo-ban-20 (Synthetic pesticide) with the target protein cytochrome b from *C. maculatus* .

Ramachandran Map calculations computed with the PROCHECK program. The phi and psi distribution of the Ramachandran Map generated by non glycine and non proline residues are depicted in Fig.1.B. Ramachandran plot indicated that no residues have phi/psi angles in the disallowed regions and hence the quality of the model is acceptable. The percentage of residues in the "core" region of modeled protein was found to be satisfactory.

Ligand Selection

The active components present in the plants are generally responsible for their insecticidal activity. The qualitative and quantitative analysis of ethyl acetate extract of *G. lotoides* through GC-MS revealed the presence of different alcohols, hydrocarbons, esters, ketones and other compounds as listed in Table.1. GC- MS chromatogram and analysis- percent peak area of *G. lotoides* ethyl acetate leaf extract shown in Fig. 2. The compound phytol is a diterpene is identified as the major metabolite in the ethyl acetate extracts of *G. lotoides*, therefore, our findings suggest that phytol might be one of the responsible metabolites for the insecticidal activity.

The structure of the best bioactive compound (Phytol) from GC-MS, downloaded from pubchem is depicted in Fig. 3.

Molecular docking

The docking simulations in the active sites of *C. maculatus* Cytochrome b were performed by the Auto dock program, which has been shown to successfully reproduce experimentally observed binding modes in terms of lowest docking energy. The target protein structure Cytochrome b was docked with bio active compound from *G. lotoides* while synthetic pesticides pirimiphos-methyl (Primo-ban-20) which provided excellent results as were seen by the least values of the binding energy. Table.2. displays the corresponding binding energy values of the docked complexes. The best possible binding modes of selected ligand with active sites of target protein were displayed in Fig.4. a and b as visualized using Pymol tool v 1.3[28].

Fig.4(a) shows the result of docking analysis of *C. maculatus* Cytochrome B with Phytol; it showed the binding site of the protein and Ligand PHE 142/HN, TRP`138/O. Fig. 3(b) shows the result of docking analysis of *C. maculatus* Cytochrome B with Pirimiphos-methyl and showed the binding site of the protein and Ligand HIS`48/HD1.

CONCLUSION

The current study was undertaken in order to study the binding potential of cytochrome b plant derived phytochemical Phytol. The result shows that the Phytol binds effectively to the receptor protein cytochrome b with the docking energy of -3.15, higher than the control pesticide used to control *C. maculates.* These results suggest use of Phytol as an effective pesticide for controlling *C. maculates* but these results were conformed to the *in vitro* studies.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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