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Molecular docking studies of acetate-succinate CoA-transferase of *Ascaris lumbricoides* with a few phytochemicals and anthelmintics

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ABSTRACT

Mitochondrial acetate production is very much essential for the energy metabolism of the parasite Ascaris lumbricoides. Acetate formation is a pre-requisite for the fatty acid synthesis in Ascaris mitochondria. The acetate is formed from acetyl-CoA by the enzymatic activity of acetate-succinate CoA-transferase (ASCT). The enzyme ASCT is not present in their host mammals. This provides an opportunity for identifying ASCT as a new drug target to control ascariasis using in silico methods. The molecular structure of ASCT is not determined experimentally and so it is not available in RCSB Protein Data Bank. Modeller9v2 was used for homology modeling of the target protein ASCT. The phytochemicals alliin, allicin, andrographolide, decursin and the existing drugs for Ascaris infection, albendazole and mebendazole, were docked on the target protein ASCT to study the inhibitory efficiency of the ligand. Decursin showed the highest docking score followed by mebendazole. The results discussed may serve as the initiative for targeting ASCT with natural resources and anthelmintic drugs.

Keywords: *Ascaris lumbricoides*, acetate-succinate CoA-transferase, phytochemicals, anthelmintics, docking.

INTRODUCTION

Nematode infections in livestock and human population are major cause for a majority of neglected tropical diseases (NTD). Ascariasis, caused by the parasite *Ascaris lumbricoides* (round worm) is considered to be an NTD by the World Health Organization (WHO). The target communities of this infection are the World's poorest people living in impoverished environment of tropical and subtropical climates and the most vulnerably affected are the children in the age group of 3 to 8 years. Severe infections can cause intestinal blockage and impair growth in children. In 2012, WHO presented a roadmap to fight against NTD's and sets the target as 2020 for their eradication.

Even though the rate of morbidity due to ascariasis is low, it can result in intestinal symptoms, malnutrition and weakness develops over time which has a direct impact on childhood development like impairing learning ability and cognitive development; it also triggers vitamin A deficiency; all these suggest for a regular deworming schedule in school children [1]. Worms cannot be eradicated from the environment we live in but control measures are a must to limit infections in the affected area. A major control program on helminth infections is mass drug administration (MDA) with benzimidazoles based on the prevalence level. Other control strategies include proper sewage disposal, improved sanitation conditions and awareness on health education. A review and meta analysis of the efficacy of anthelmintic drugs revealed that only a few drugs viz. albendazole, mebendazole, levamisole and pyrantel pamoate

are effective against *A. lumbricoides*, *Trichuris trichiura* and *Ancylostoma duodenale* [2]. Geerts and Gryseels reviewed the reports on drug resistance in human helminths and the methods of identifying the anthelmintic resistance in livestock and human [3]. Vercruysee *et al*. addressed the issue of anthelmintic resistance in livestock and a warning against anthelmintic resistance in human; this review also explained the tools available for resistance monitoring [4]. The alarming resistance of parasites to the available anthelmintics proposes the need for the development of new drugs with different mechanism of action.

Ascaris is a gastrointestinal parasite of mammals that excrete acetate as one of the major end products of anaerobic malate dismutation reaction. Parasitic helminths reside in environments of low oxygen tension. They have modified their metabolic pathways of ATP synthesis that are independent of oxygen as the terminal electron acceptor. Acetate is an important end product in the energy metabolism of the parasite. The CoA moiety of acetyl-CoA is transformed to succinate yielding acetate as an end-product of the malate dismutation reaction [5, 6].

Four different pathways are identified for the production of acetate from acetyl-CoA in prokaryotes and eukaryotes. In anaerobically functioning mitochondria of parasitic helminths, the conversion of acetyl-CoA to acetate is by the activity of the enzyme acetate-succinate-CoA transferase (ASCT), the ASCT reaction yields succinyl-CoA for ATP synthesis via succinyl-CoA synthetase (SCS) [7].The parasite specific enzyme ASCT catalyses the reaction in a succinate dependent manner.

Acetyl-CoA + succinate $\longrightarrow$ acetate + succinyl-CoA

The succinyl-CoA synthesised by the activity of ASCT is subsequently recycled to succinate by SCS generating ATP by substrate level phosphorylation. SCS is also a Kreb's-cycle enzyme found in acetate producing organisms as well as in most aerobically functioning organisms. SCS catalyses the irreversible reaction,

Succinyl CoA + ADP + Pi $\longrightarrow$ succinate + ATP + CoA

ASCT activity is not present in the mammalian host and this enzyme may be an attractive target for the development of novel antiparasitic drugs. ASCT has been identified as a drug target by elementary mode analysis on the energy metabolism of the parasite *A. lumbricoides* [8]. In the present study an *in-silico* analysis on the inhibition of ASCT by a few phytochemicals and existing anthelmintics has been discussed.

In Brazil, a study on children infected with *A. lumbricoides* evaluated the effectiveness of *Allium sativum* against ascariasis [9]. Coon and Ernst reviewed the pharmacological activities of *Andrographis paniculata* and suggested it as a safe and efficacious treatment for the relief of symptoms of uncomplicated upper respiratory tract infection [10]. Shiomi *et al*. suggested that decursin and decursinol angelate are inhibitors of NADH dependent fumarate reductase of *A. suum* [11]. The main drugs used to treat human nematodes are mebendazole, albendazole, pyrantel pamoate and levamizole. The mode of action of Benzimidazoles viz., albendazole and mebendazole is that it binds to the structural protein tubulin which interferes with the polymerization of microtubuli. Levamizole and the related anthelmintic pyrantel are cholinergic agonists with a selective action on nematode receptors.

In the present investigation the phytochemicals namely alliin, allicin, andrographolide, decursin and the broad spectrum of anthelmintics albendazole and mebendazole, were docked on the target proteins ASCT of the parasite *Ascaris* using bioinformatics tools to study the inhibitory efficiency of the ligand on the protein. The structure of the ligand molecules used in docking has been retrieved from NCBI-PubChem [12]. AutoDock was used to study the binding of the ligands with the receptor ASCT.

MATERIALS AND METHODS

DATABASES

NCBI-Protein: The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI protein database is a collection of protein sequence records from various sources such as PDB, Swiss-Prot, GenPept, RefSeq, PIR and PRF.

Pubchem Database: The PubChem Substances Database contains descriptions of chemical samples from a variety of sources and links to PubMed citations, protein 3D structures and biological screening results that are available in

PubChem BioAssay. If the contents of a chemical sample are known, the description includes links to PubChem Compound www.ncbi.nlm.nih.gov › NCBI › Chemicals & Bioassays.

TOOLS
3D-STRUCTURE VISUALIZATION:
RASMOL
RasMol is a molecular graphics program used for the visualisation of proteins, nucleic acids and small molecules. The loaded molecule can be shown as wireframe bonds, cylinder 'Dreiding' stick bonds, alpha-carbon trace, space-filling (CPK) spheres, macromolecular ribbons (either smooth shaded solid ribbons or parallel strands), hydrogen bonding and dot surface representations. Available at: http://rasmol.org/

CHEM SKETCH
ACD/ChemSketch is an advanced chemical drawing and graphics package from ACD/Labs. It is the accepted interface for the industries best NMR and molecular property predictions, nomenclature, and analytical data handling software. It was developed to help chemists quickly and easily draw molecules, reactions, and schematic diagrams, calculate chemical properties, and design professional reports and presentations.

PDBSUM
The active site of the protein is the binding site where catalysis occurs. The binding sites of the protein are usually found in cavities or on the polar surface. The ligand binding sites of the template and target were identified using UniProt database [13]. PDBsum provides information of amino acids that have interactions with the ligand as a LIGPLOT [14]. LIGPLOT is a two dimensional representation of protein ligand interaction from a standard PDB file input. PDBsum also provides a wiring diagram that shows amino acid interactions with the particular ligand. There is a correlation between the wiring diagram and LIGPLOT as they show the same amino acid interactions with the ligand binding sites. The wiring diagram gives the active sites of the template.

AUTODOCK
Auto Dock is a suite of automated docking tools. The software is used for modeling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structures. It uses Genetic Algorithms for the conformational search and is a suitable method for the docking studies. The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evaluation. Auto Dock tools is used to prepare, run and analyze the docking simulations, in addition to modeling studies. Auto Dock is the most cited docking software because it is very fast, it provides high quality predictions of ligand conformations and good correlations between inhibition constants and experimental ones. Auto Dock has also been shown to be useful in blind docking, where the location of the binding site is not known [15].

PYMOL
PyMOL (PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC) is one of a few open-source visualization tools available for use in structural biology. The Py portion of the software's name refers to the fact that it extends, and is extensible by the Python programming language. PyMOL uses OpenGL Extension Wrangler Library (GLEW)

RESULTS AND DISCUSSION

Homology modeling of ASCT:
ASCT of *Ascaris* is not studied experimentally; the sequence of ASCT of *Fasciola hepatica* is retrieved from NCBI database. The ASCT sequence of *Fasciola hepatica* with the length of 478aa and GenBank Accession No. ACF06126.1 is considered for building the theoretical model. Initially it was ascertained that the three dimensional structure of ASCT was not available in database, hence an attempt had been made in the present study to determine the three dimensional structure of ASCT. In the absence of an experimentally determined structure, homology modeling can sometimes provide a useful 3D model for a protein that is related to at least one known protein structure. Homology modeling predicts the 3D structure of a given protein sequence (target) based primarily on its alignment to one or more proteins of known structure (templates). The prediction process consists of fold assignment, target-template alignment, model building, and model evaluation. The FASTA sequence of the query protein was retrieved from NCBI Entrez sequence search. Following BLAST [16] run, suitable template sequence has been identified. The 3D-structure of query protein was predicted by homology modeling using Modeller9v2

[17]. The rough model generated was validated using the Ramachandran plot [18] obtained from the PROCHECK server [19]. The protein was modeled using the template, PDB ID 2OAS, Chain A, crystal structure of 4-Hydroxybutyrate coenzyme A transferase (AtoA) in complex with CoA from *Shewanella oneidensis* with 49% similarity and a resolution of 2.4 Å. The pair wise sequence alignment of the target and template is shown in Fig. 1. The modeled three dimensional structure of ASCT is shown in Fig.2. (Pink color coil indicates Alpha Helix, Yellow color arrows indicate beta sheets, and blue & white color indicates turns and coils). The Ramachandran plot statistics are shown in Fig. 3.

Ligand Preparation:

The ligand structures were taken from Pubchem compound database and the two-dimensional structures of Allicin, Alliin, Andrographolide, Decursin, Albendazole, and Mebendazole were drawn using ACD/ChemSketch and saved in MDL-MOL. The mol format is converted to babel molecular converter PDB format using open-babel molecular converter.

Chain A, Crystal Structure Of 4-Hydroxybutyrate Coenzyme A Transferase (Atoa) In Complex Witl
Target Sor119.

Sequence ID: pdb|2OAS|A Length: 436 Number of Matches: 1

▶ See 1 more title(s)

Range 1: 11 to 422 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
380 bits(977)	3e-127	Compositional matrix adjust.	205/419(49%)	270/419(64%)	12/419(2%)

```
Query  56   ETFGFLKDGANVFIHGGAATPSLLIKELYEYVMSKNLKDIKLFHIHTEGPYPFNDAE--G  113
            E    ++ G  ++ H   ATP +L+  L ++ ++  L +I L  +HTEG    +     G
Sbjct  11   EAVSLIRSGETLWTHSXGATPKVLLDALAKHALT--LDNITLLQLHTEGAESLSHPSLLG  68

Query  114  HFRSTSLFTGGNCRKAIQEGRADYTPIFLSEIPLLFRRKHVQLDLALINVTPPDKHGFCS  173
            H R    F G   R  +Q G ADY PIFLSE+P LFR    ++D A+I V+PPDKHG CS
Sbjct  69   HLRHRCFFGGVPTRPLLQSGDADYVPIFLSEVPKLFRSGEQKIDTAIIQVSPPDKHGXCS  128

Query  174  LGPSVDVTRAAIQNATHIVAQVNDQLPLTRGDASIHFSNLTVM--RAGSQPCHEMSPRPA  231
            LG SV+ T AA Q A  I+A +N Q P T GD  IH      +  ++ S P H  +   A
Sbjct  129  LGISVEATLAACQVAGKIIAHINPQXPRTHGDGFIHIDRFAAVYEQSASLPIHSFATGDA  188

Query  232  SEVEDKIGQIIAENLVEDGATLQTGIGAIPDAVLSKLTNHKNLGVHTEMFSDGVVQLVQL  291
              V   IGQ +AE LV DG  LQ GIGAIPDAVLS LT HK+LGVHTE+FSDG++QLV+
Sbjct  189  --VSLAIGQHVAE-LVRDGDCLQXGIGAIPDAVLSCLTGHKDLGVHTELFSDGILQLVEK  245

Query  292  GAITNAYKKLRPGKVVSSFVVGTRKVFDFLDNNPMVDMCDVAWVNSPVVIAQNPKPVAIN  351
            G I N  K+  PGK+V+ F +G++K++D++D+NP V   D+  VN   +I +NP   AIN
Sbjct  246  GVINNTKKRFYPGKLVTGFALGSQKLYDYVDDNPAVIFXDIEQVNDTSIIRKNPNVXAIN  305

Query  352  SCIEIDITGQVSSDSIGTTIYSGFGGQVDFLRGAAVSLDGQGKPIIAVPSVTKRNE-TKI  410
            S +++D+TGQV +DSIGT IYSG GGQ DF+RGA +S +G G+ +IA+PS       ++I
Sbjct  306  SALQVDLTGQVCADSIGTKIYSGVGGQXDFIRGAGLS-EG-GRSVIALPSTAAGGRISRI  363

Query  411  VPHLKLGGGVVTTRAHVHYVVTEYGIAYLFGKNLRQRAHALIQIAHPDHRESLEKAAFD  469
               L  G GVVTTRAHVHY+VTEYG A L G++LR+RA ALI IAHPD RE L + AF+
Sbjct  364  ASVLSPGAGVVTTRAHVHYIVTEYGAANLKGRSLRERAQALINIAHPDFREQLSRDAFE  422
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Fig. 1: Target-template allignment

Fig.2. 3D structure of the modeled receptor ASCT

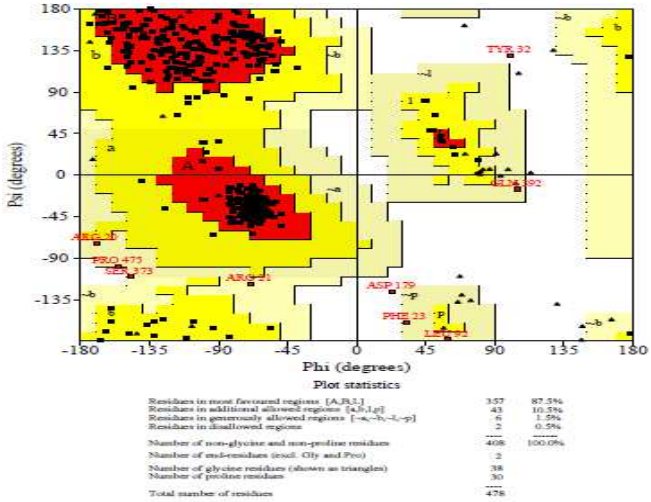
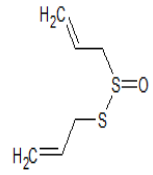
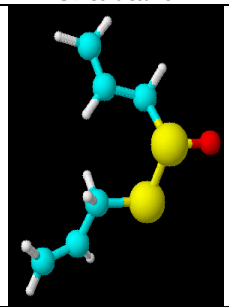
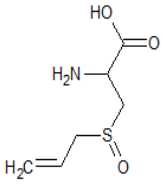
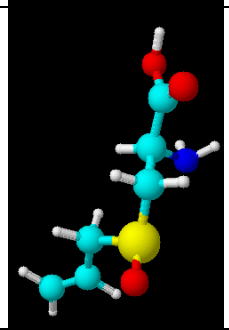
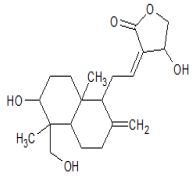
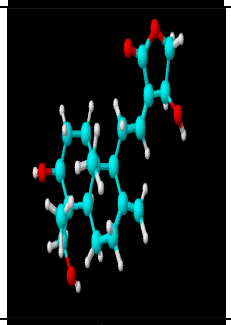
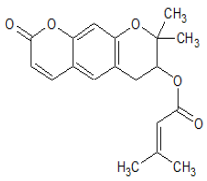
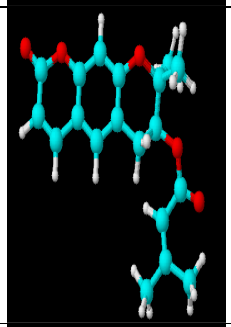
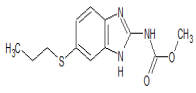
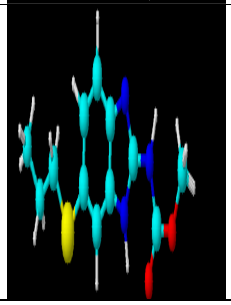
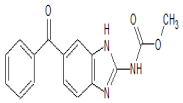
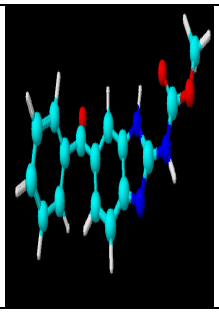


Fig.3. Ramachandran plot of modeled protein

The compounds that can act as effective therapeutic agents satisfy the Lipinski's rule of 5, formulated by Christopher A. Lipinski in 1997 [20]. It is a rule of thumb to evaluate the drug likeness property of the ligand molecule. It states that an orally active drug cannot have a molecular weight of more than 500 Daltons, has not more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors and partition co-efficient log P less than 5. The 2D and 3D structures and the Lipinski's rules of 5 were shown in Table 1.

Table 1: Structure and Lipinski's rule of 5 for Ligands

Ligands	2D structure	3D structure	Properties	XLogP3
Allicin			Molecular weight (g/mol): 162.273 Molecular Formula: $C_6H_{10}OS_2$ Hydrogen bond donor: 0 Hydrogen bond acceptor: 3 Lipinski's rule: Yes	1.3
Alliin			Molecular weight (g/mol): 177.221440 Molecular Formula: $C_6H_{11}NO_3S$ Hydrogen bond donor: 2 Hydrogen bond acceptor: 5 Lipinski's rule: Yes	-3.5
Andrographolide			Molecular weight (g/mol): 350.4492 Molecular Formula: $C_{20}H_{30}O_5$ Hydrogen bond donor: 3 Hydrogen bond acceptor: 5 Lipinski's rule: Yes	2.2
Decursin			Molecular weight (g/mol): 328.3591 Molecular Formula: $C_{19}H_{20}O_5$ Hydrogen bond donor: 0 Hydrogen bond acceptor: 5 Lipinski's rule: Yes	3.9
Albendazole			Molecular weight (g/mol): 265.3314 Molecular Formula: $C_{12}H_{15}N_3O_2S$ Hydrogen bond donor: 2 Hydrogen bond acceptor: 4 Lipinski's rule: Yes	2.9

Mebendazole			Molecular weight (g/mol): 295.29272 Molecular Formula: $C_{16}H_{13}N_3O_3$ Hydrogen bond donor: 2 Hydrogen bond acceptor: 4 Lipinski's rule: Yes	2.8

Active site prediction:

The active sites of the target (ASCT) have been identified by the homology model predicted for the target and the template. The ligand binding site of the template sequence identified as CoA from the UniProt database is shown in Fig.4. The active sites of the target protein were PHE121, GLU279, PHE310, SER352, CYS353, ILE354, GLU355, SER364, ILE367, TYR372, SER373, GLY374, PHE375, GLY376, GLY377, GLN378, LYS409, THR403 and LYS404.

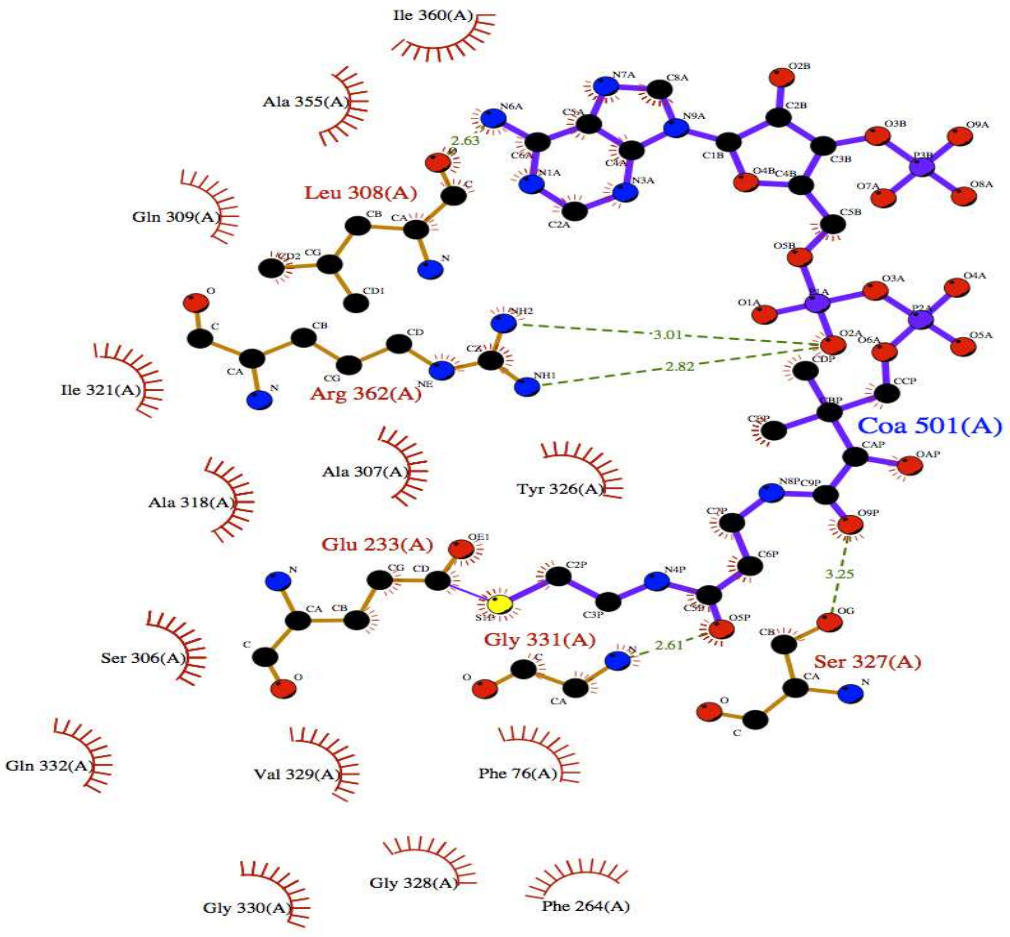


Fig. 4 Ligand binding site of the template identified from UNIPROT database

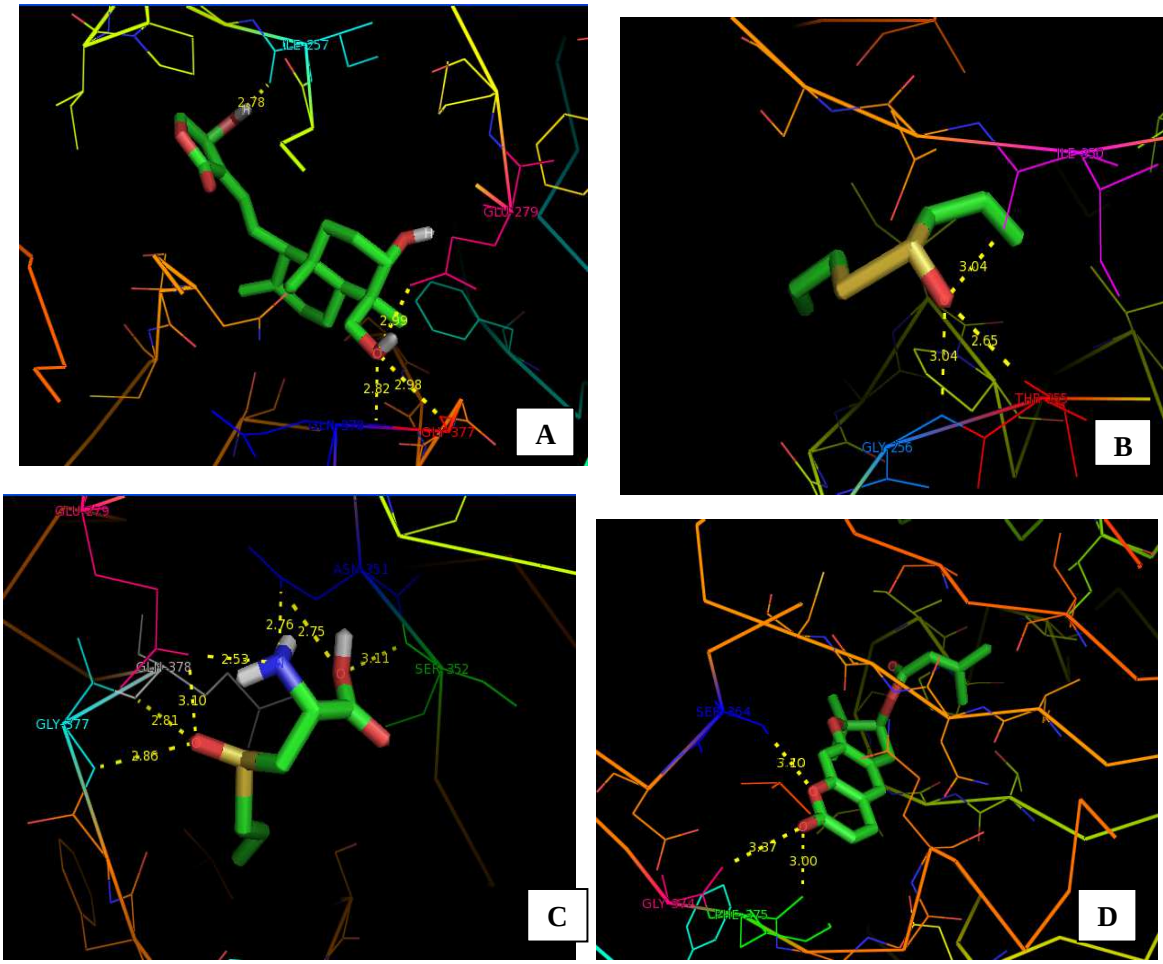
Docking Analysis

Docking is used to find the exact binding conformation and orientation of the ligand molecule into the active site of the protein. The six compounds namely Allicin, Alliin, Andrographolide, Decursin, Albendazole and Mebendazole

were docked against ASCT using Auto-Dock Tool 4.0, an automated docking tool. The docking process involves four main steps, (i) Protein preparation (ii) Ligand preparation (iii) Grid preparation and (iv) Docking. The Lamarckian genetic algorithm has been used as the search algorithm to search for the best conformers. The initial population size was set randomly as 150 individuals and ten generations was set for each genetic algorithm run and the maximum number of energy evaluations was set to 2,500,000. The grid box size was set as to include all the active site residues present in rigid macromolecules. The spacing between grid points was 0.375Å. The grid box was centered at 27.616 Å x 34.41 Å x 76.132 Å and the dimensions of the grid box have been set as 60,72, 90 (X,Y,Z co-ordinates) so as to include all the active site residues.

Docking studies showed that all ligands chosen for analysis possessed a least binding affinity with the target protein ASCT. The protein ligand interactions were studied in terms of minimum binding energy (Kcal/mol) and the number of hydrogen bonds formed with active site residues. The docking interactions of the six ligands and the protein ASCT were visualised using PyMOL viewer and shown in Fig.5. The final docked confirmation obtained for the different ligands based on the binding energy, number of hydrogen bonds formed, bond distance and the interacting residues were shown in Table 2.

Decursin and Mebendazole show a least binding energy with the docking score of -7.40 Kcal/mol (forms three hydrogen bonds with SER364, GLY374, and PHE375) and -7.33 Kcal/mol (forms three hydrogen bonds with GLU279 and HIS101), respectively, when docked against ASCT. Alliin and Allicin form a maximum number of seven and three hydrogen bonds, respectively, with the docking score of -5.17 and -4.63 Kcal/mol.



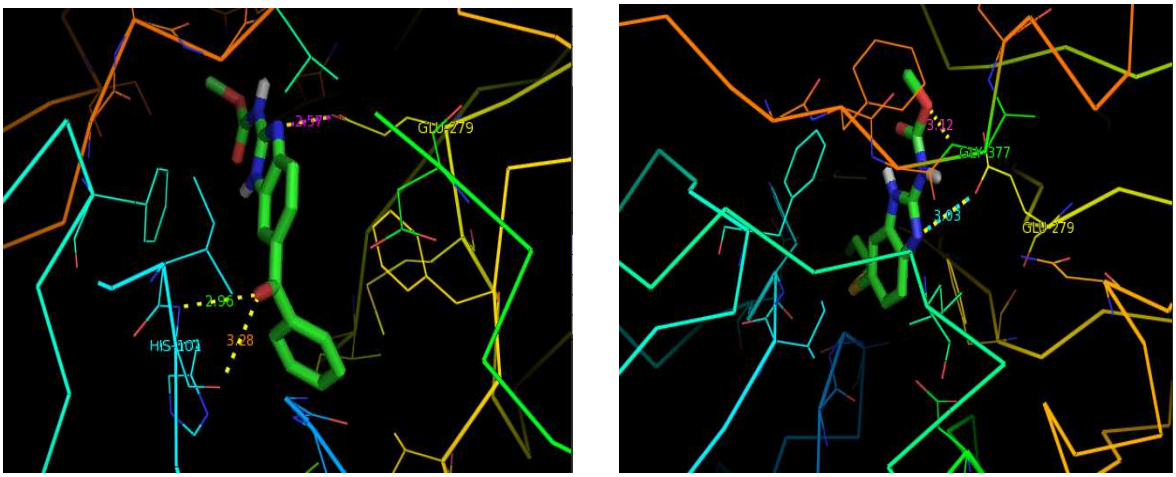


Fig. 5 Docking interactions of ASCT of *A. lumbricoides* with (A) allicin, (B) alliin, (C) andrographolide, (D) decursin (E) mebendazole, (F) albendazole

The length of the hydrogen bonds formed with interacting residues for all the ligands, which shows that the bonding was good. Most of the key residues shown in the Table 2 are the active site residues of the target protein predicted by PDBsum. Based on the docking score all the ligands have docking interactions with the protein ASCT. The compounds with anthelmintics activity was chosen as ligands in this study have different mode of action. Decursin is also shown to target the NADH-fumarate reductase of Ascaris [11]. This *in silico* molecular docking study shows that all the four phytochemicals alliin, allicin, andrographolide and decursin inhibits the drug target ASCT.

Table 2: AutoDock interaction of the ligands with ASCT of *A. lumbricoides*

Ligand	Docking score (kcal/mol)	Number of hydrogen bonds	Key residues	Distance (Å)
Allicin	-4.63	3	THR255 (OG1···O) GLY256 (N···O) ILE350 (O···O)	2.65 3.04 3.04
Alliin	-5.17	7	GLU279 (OE2···N) GLU279 (OE2···O) ASN351 (OD1···O) ASN351 (OD1···N) SER352 (N···O) GLY377 (N···O) GLN378 (N···O)	2.53 3.10 2.75 2.76 3.11 2.86 2.81
Andrographolide	-6.04	4	ILE257 (O···N) GLU279 (OE2···O) GLY377 (N···O) GLN378 (N···O)	2.78 2.99 2.98 2.82
Decursin	-7.40	3	SER364 (OG···O) GLY374 (O···O) PHE375 (O···O)	3.10 3.37 3.00
Albendazole	-6.54	2	GLY377(N···O) GLU279(OE1···N)	3.12 3.03
Mebendazole	-7.33	3	HIS101(N···O) HIS101(ND1···O) GLU279 (OE1···N)	3.28 2.96 2.57

CONCLUSION

In recent years, biology of parasites has been studied extensively by the researchers. Studies on the physiology and biochemistry of helminth parasites paved way for identifying new drug targets that are unique to each parasite. ASCT is a parasite specific enzyme and drugs inhibiting ASCT are selectively toxic to the parasite with no effect on the host. Benzimidazoles namely albendazole and mebendazole are the drugs of choice for ascariasis and this study shows that benzimidazoles have a potent inhibitory effect on the parasite ASCT. Phytochemicals namely alliin,

allicin, andrographolide, and decursin show good inhibitory effects against the drug target ASCT and utilized least docking energy and forms more hydrogen bonds than the benzimidazoles which is the commonly used drug for ascariasis. The *in silico* docking studies reveal that ASCT could act as a potent drug target for *Ascaris* infections which could be further validated by *in vivo* and *in vitro* studies and these phytochemicals may act as potential and natural therapeutic agents to treat ascariasis.

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