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DOCKING ANALYSIS OF ENDOPHYTIC FUNGAL METABOLITES REPORTED FROM PHARMACEUTICALLY IMPORTANT PLANTS AGAINST ANCYLOSTOMA-SECRETED PROTEIN (SAP-1) OF THE NEMATODE NECATOR AMERICANUS- A HUMAN PATHOGEN

Kalyanaraman Rajagopal, Annie Absala Trace and Radhai Rajendran
School of Life Sciences, Department Of Biotechnology, Vels University (VISTAS),
Pallavaram, Chennai-600117.
Email: ammaaappa@gmail.com

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Abstract

Ancylostoma-secreted protein 1 (ASP-1) is the most important protein secreted by infective hookworm *Necator americanus* in host. It is a major protein secreted during activation and play a important role in hookworm adaptation to parasitism. In the present study, the identification of potential inhibitors for ASP-1 was carried out. Endophytic fungai produce various metabolites which are active against human and plant pathogens which include nematodes also. About 16 biologically active endophytic fungal secondary metabolites were analyzed for its drug likeliness based on Lipinski's rule of five. Out of 16 compounds studied, only 5 compounds are able to satisfy the Lipinski's rule namely heptelidic acid, isocoumarin, peramine, phomadecalin E , and phomenone. These 5 compounds were subjected to docking study using AutoDock 4.0. The molecular docking studies revealed that heptelidic acid, isocoumarin, phomadecalin E, phomenone, and peramine bind effectively to the active site region of ASP-1with a binding energy of −15.84, -14.62, -11.16, -6.54, -6.11 respectively. The above 5 compounds showed significant inhibition against the nematode protein ASP-1 are suggested to be better lead molecules for drug development in future against *Necator americanus* a human pathogen.

Keywords: ASP-1, Docking, Endophytic Fungi, Hookworm, Human Pathogen, Secondary Metabolite.

Introduction

Necator americanus is human hookworm [1].It is transmitted through the soil about 740 million human hookworm infections occur worldwide every year, *N. americanus*is considered to be the most common infections cause of anemia [2]. It can be transmitted through penetration of the skin [3]. During pregnancy causes a neonatal

prematurity and low birth weight[4]. Major proteins secreted by the infective larval stage hookworms *Ancylostoma* secreted proteins (ASPs), which are characterized by one or two CAP (cysteine-rich secretory protein) and the members of the PR-1 subfamily .There are two types of ASP have been isolated from larval nematodes single PR-1 domain (Na-ASP-2) or two PR-1 domains (Na-ASP-1). Na-ASP-1 is a 406-amino-acid protein with molecular weight of 43.9 kDa[5]. *Ancylostoma* secreted protein (ASP) in infectivelarva L3 stage this protein binds to human NK cells, resulting in IFN-γ production induced intestinal inflammation[6].Docking studies are used to predicts the preferred orientation of one molecule to another when bound to each other to form a stable complex. It is used to find the binding affinity between two molecules protein and ligand. Fundamental screening of the Ligand-protein interaction for their binding affinity was carried out using AutoDock and the results that include the understanding H-bonding and hydrophobic interactions were analyzed using discovery studio a programme to generate schematic diagrams of protein ligand interactions. Ligand property was predicted by using Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules[7]. In the present study, we have studied the drug-likeness of secondary metabolites from the endophytic fungi using Lipinski's rule of five and the binding mechanism of the compounds with nematode *Necator americanus* protein ASP-1 using molecular docking studies for drug development.

Materials and Methods

Preparation of protein structure

The three-dimensional (3D) structure of the ASP-1 protein was taken from the Protein Data Bank (PDB) (www.rcsb.org) the structure is given in (Fig.1.).The PDB ID of ASP-1 protein is 3NT8. The active site region of the ASP-1 given in (Table.3).

Fig. 1: ***Necator americanus*****(Ancylostoma secreted protein-1) PDB ID: 3nt8 selective inhibitor**

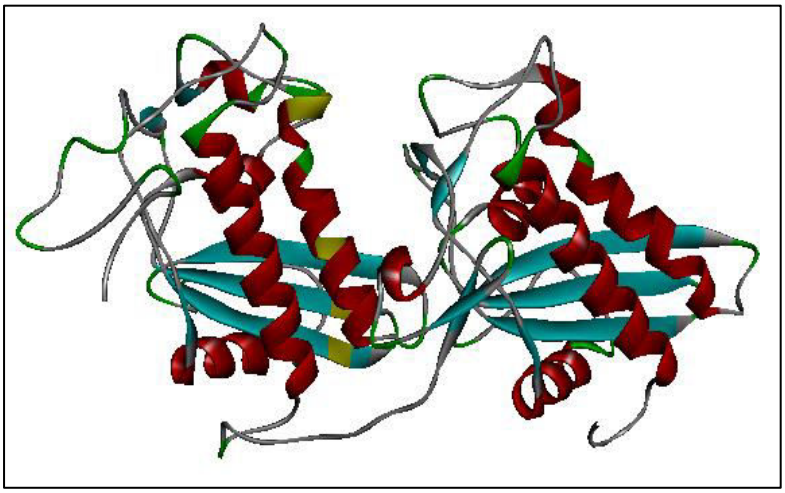


Table 3: ***Necator americanus*** **PDB ID: 3nt8 selective inhibitor showing the active site region of ASP-1.**

LYS67,ASN68,LYS140,TYR154,SER155,GLU156,THR158,LYS159,ASN178,GLY179, VAL180,ILE183,THR184,ASN185,GLN186,PRO187,MET188,TRP189,GLU190,SER202, THR203,TYR204,LYS205,ASN206,LYS216,GLY217,PRO218,ASP219,VAL220,GLU222,SER236,ASP239, THR240,SER243,VAL244,GLU247,PHE248,SER251,LEU256,GLU257, PRO258,PHE316,ASP317,ASN319,LYS320,LYS323,GLN324,GLN327,LEU328,ASN331,LYS334,GLU335

Docking Study Auto Dock 4.0

AutoDock combines two methods to Rapid grid-based energy evaluation and efficient search of torsional freedom. It works by Lamarckian Genetic Algorithm. ASP-1 receptor protein was docked against the five endophytic fungal secondary metabolites using Auto Dock 4.0. Endophytic fungal metabolites were docked into the active site of 3NT8 receptor protein.

Discovery studio visualizer 3.1

Discovery studio visualizer is a tool for molecular modeling study, for both small molecule, and macromolecule applications. It generates 2D receptor-ligand interaction plots and analyzes the ligand binding patterns between a protein and its bound ligands.

Metabolites from endophytic fungi

Endophytic fungi reside in the plant and help the plants by producing various chemical compounds [8,9] . In the present study 11 endophytic fungal metabolite namely (1) Heptelidic acid (2) Isocoumarin (3) Phomadecalin E (4) Phomenone (5) Peramine, (6) Trichodermin (7) Scoparasin B (8) Phomoenamide (9) Patulin (10) Isopestacin and (11) Isocochliodinol were subjected to docking studies . The structures and the physiochemical properties of these compounds were taken from the PubChem database. Lipinski's rule of five parameters such as molecular weight, log P, and number of hydrogen bond donors, hydrogen bond acceptors and molar refractivity were taken from the Lipinski Rule of Five tool for the endophytic fungi secondary metabolites (Table 1).

Table-1: Lipinski's properties of the endophytic fungal secondary metabolite.

S.NO	NAME OF COMPONDS	MASS (<500 Da)	LOG P (<5)	H-DONAR(<5)	H ACCEPTOR(<10)	MOLAR REFRACTIVITY (40-130)
a	Patulin	152	-0.1	0	4	31.91
b	Isopestacin	288	2.5	7	5	79.6
c	Isocoumarin	146	1.8	0	2	40.8
d	Isocochliodinol	502	6.4	4	4	148.6

e	Trichodermin	284	2.2	0	4	71.7
f	Phomenone	256	0.7	2	4	61.0
g	Phomadecalin E	264	1.9	4	4	68.7
h	Heptelidic acid	276	0.6	0	5	70.9
i	Scoparasin B	531	1.0	3	9	126.4
j	Peramine	243	0.8	3	5	70.3
k	Phomoenamide	278	1	6	0	0

Preparation of ligand structures

Chemically intelligent drawing interface freeware developed by Advanced Chemistry Development, Inc., (http://www.acdlabs.com) was used to build the structure of the ligands. Using draw mode of Chemsketch, the ligands were created and the three dimensional optimizations were done. Out of 11 compounds studied using Lipinski's rule only six compounds were passed in Lipinski's rule of five used for docking studies the metabolites studied are Heptelidic acid, Isocoumarin, Phomadecalin E, Phomenone, Trichodermin and Peramine.

Results

The protein ASP-1 contains 25% of Alpha helix and 12% of Beta turn and 39% of Random coil shown in (Fig.1). Endophyti**c** fungal secondary metabolite studied for Lipinski's rule of five like Molecular weight should (<500 Dalton) ,log p(<5),H bond donar (<5), H bond acceptor (<10) and molar refractivity (between 40-130) indicates the metabolite to pass Lipinski's rule and qualify for docking study . Based on Lipinski's characteristic the metabolite Heptelidic acid, Isocoumarin, Phomadecalin E, Phomenone, Trichodermin and Peramine are used for docking analysis. The docking analysis carried out using Autodock 4.0 The result of Lipinski's rule of five Molecular weight, Log P, Number of H bond donar , H bond acceptor and and molar refractivity are tabulated in (Table-1). Out of the 11 endophytic fungi metabolite analysed only 6 metabolites satisfies the lipinski's rule of five for drug likeliness .The other 5 compounds do not follow the lipinski's rule where not considered for docking analysis . Trichodermin even though it passed lipinski's rule it does not show proper result in docking analysis. The structure of drug likeliness metabolites from endophytic fungi are shown in(Fig. 2). The binding energy for the five metabolites with ASP-1 using Autodock 4.0 given in (Table .4). The docking analysis shows that the ligands bind to the active domain site of ASP-1 protein with significant energy in the same hydrophobic pockets. The docking analysis of selected metabolites in 3 dimensional views are shown in (Fig.3a-3e).The hydrogen contacts of selected metabolite given in (Table 2). The docking scores were highest for heptelidic acid with −15.84, isocoumarin with -14.62 and

phomadecalin E with -11.16, phomenone with -6.54 and peramine with-6.11 respectively and the binding mode of endophytic metabolites with ASP-1 receptor protein.

Fig-2: The 3D structures of compounds (a) Heptelidic acid, (b) Isocoumarin, (c) Phomadecalin E, (d) Phomenone, (e) Peramine

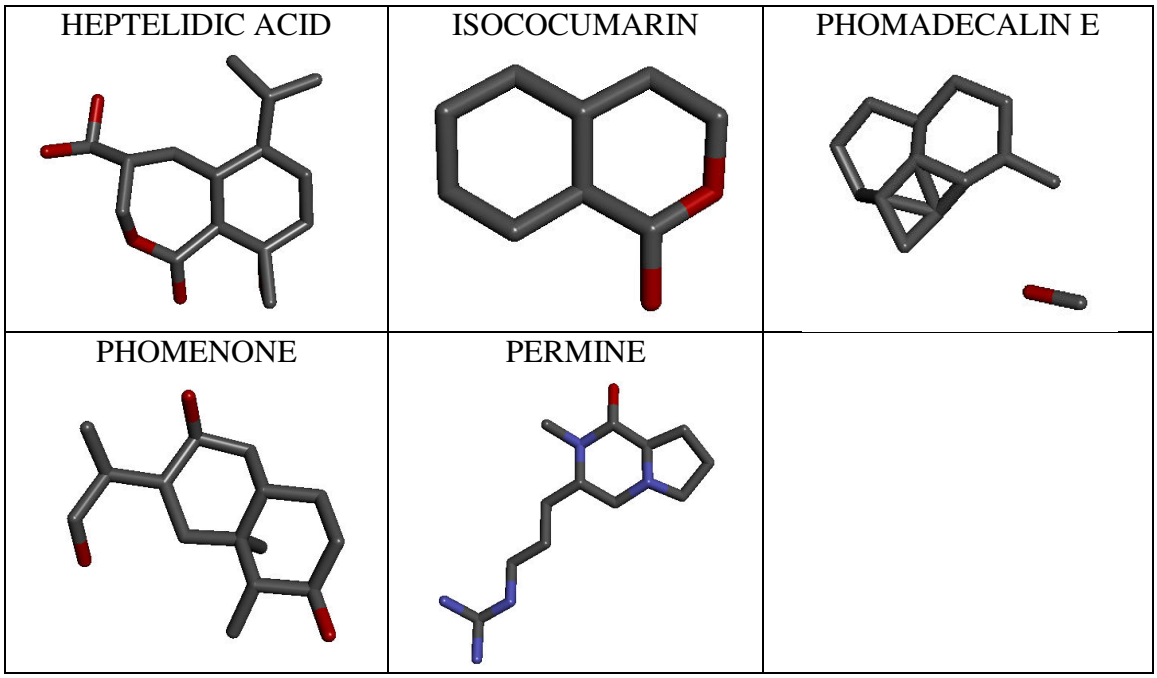


Table 4: Dock scores of the compounds.

a	Heptelidic acid	−15.84
b	Isocoumarin	-14.62
c	Phomadecalin E	-11.16
d	Phomenone	-6.54
e	Peramine	-6.11

Fig-3(a): Docking complex of Ancylostoma secreted protein-1(PDB ID: 3nt8) with Heptelidic acid.

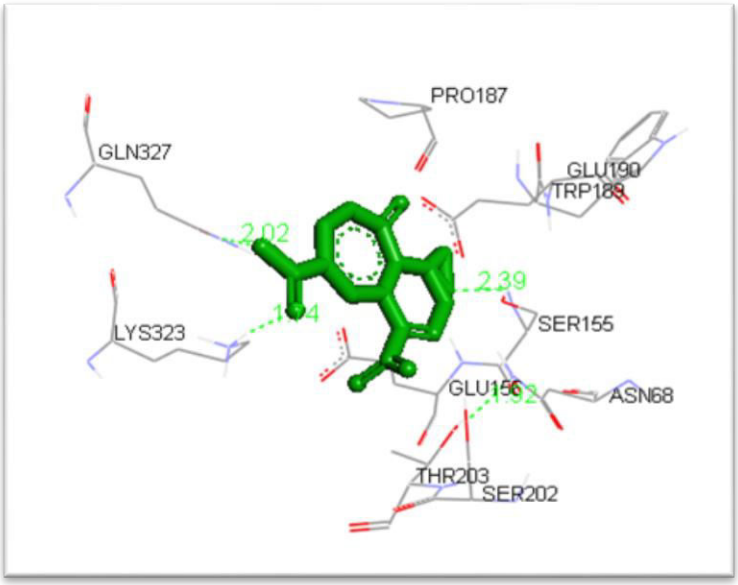


Fig-3(b): Docking complex of Ancylostoma secreted protein-1(PDB ID: 3nt8) with isococumarin.

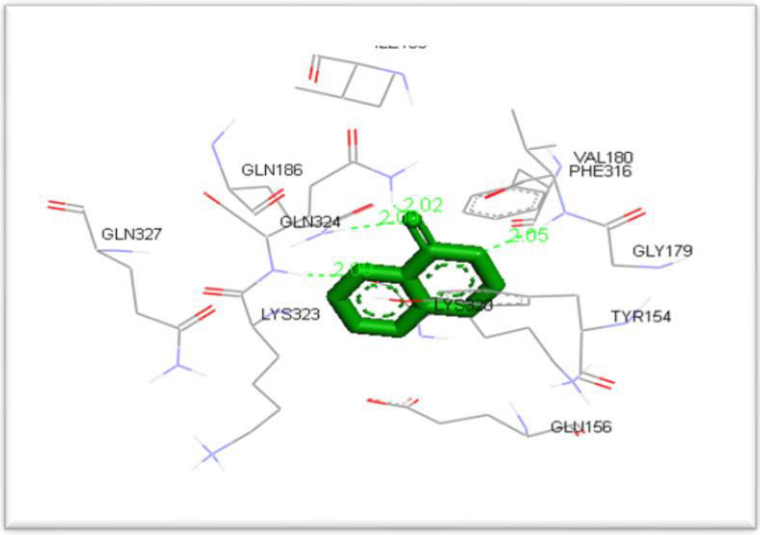


Fig-3(c): Docking complex of Ancylostoma secreted protein-1(PDB ID: 3nt8) with phomadecalin E.

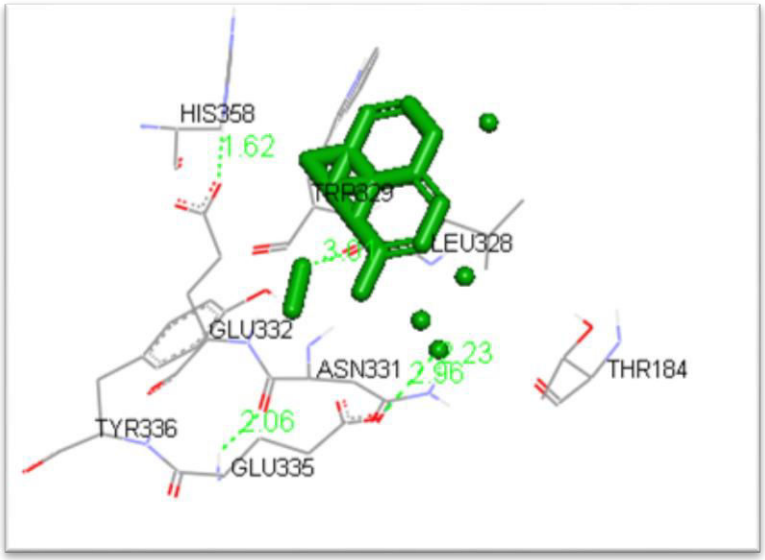


Fig-3(d): Docking complex of Ancylostoma secreted protein-1(PDB ID: 3nt8) with phomenone.

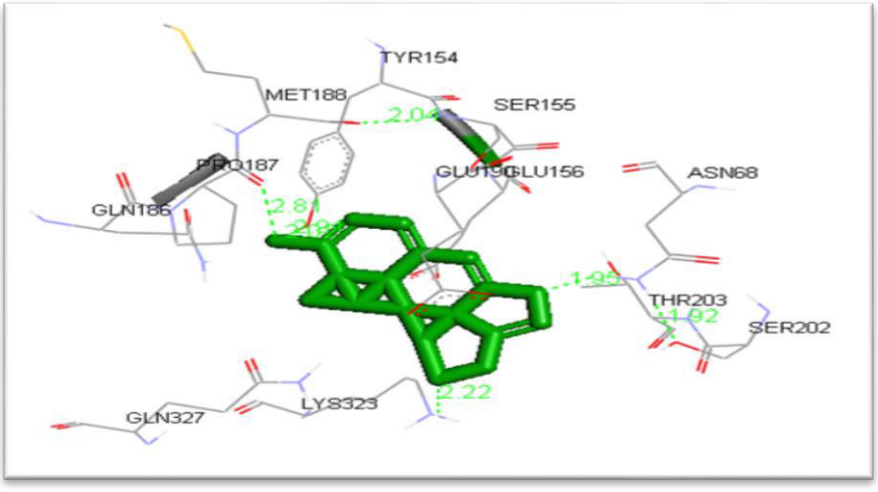
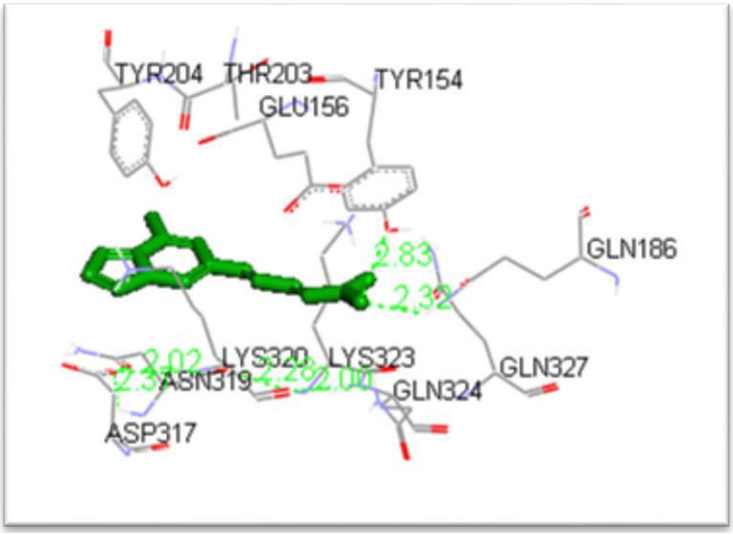


Fig-3(e): Docking complex of Ancylostoma secreted protein-1(PDB ID: 3nt8) with peramine.



Discussion

Endophytic fungal metabolites has many biological effect. However, antinematicidal property for endophytic fungal metabolite is very much limited. Several Insilco studies proved that, Secondary metabolites from endophytic fungi has got lot of anticancer, antiinflamatory, antimicrobial potential [10,11,12]. More number of Insilco studies on plant derived compounds has been done. The diverse bioactivities of endophytic fungal metabolites are less understood by insilco designing studies.

Ancylostroma secreted protein (ASPs) is a key protein during the infection of *Necator americanus* .Human hookworm induce chronic infections in their host .These parasites have developed unique mechanism to evade host immunity by producing excretory/secretory (ES) products in the host [13]. In response to this parasitic infection two proteins are secreted ASP1 and ASP2 it belong to the pathogenesis-related protein PRP superfamily [14].Due to the hookworm infection increase in the level of ASP is reported. Thus, inhibition of this protein ,will be an important mechanism to control this infection. Presently Benzimidazole, Mebendazole and Albendazole drugs being used to control hookworm infection [15].These drugs shows several side effects in hosts such as gastrointestinal symptoms diarrhea, nausea, vomiting headache, dizziness[16].The present investigation focuses on docking studies of natural secondary metabolites from endophytic fungi, for anti nematicidal property and to overcome undesirable biological side effects. The results of Insilco docking study using Autodock 4.0 shows that out of 11 endophytic fungi metabolite analyzed only 6 metabolites pass the Lipinski's rule of five for drug lightness even though 6 metabolites pass the Lipinski's rule only 5 metabolites satisfactorily bind with target protein .The 5 compounds which satisfy Lipinski's property are Heptelidic acid, Isocoumarin, Phomadecalin E, Phomenone and Peramine these 5 compounds

bind with active site of ASP-1 protein with good binding energy of −15.84, -14.62, -11.16,-6.54 ,-6.11 respectively (Table.4) . The amino acid residues with which hydrogen bonds formed for Heptelidic acid are Ser155, Lys323, Gln327; Isocoumarin forms hydrogen contacts with Gln324, Gln186, Val180; Phomadecalin E establish hydrogen contacts with Glu335, Leu338, Asn331; Phomenone forms hydrogen contacts with Lys323, Asn68, Pro187, Tyr154 and Peramine forms hydrogen contacts with Gly156, Tyr154, Gln327, Tyr203 the results given in (Table.2) are in the active site of ASP-1. Heptelidic acid and Isocoumarin binds in the binding site with more hydrogen bonds when compared to other compounds investigated in the present study. This indicates that these two compounds has better anti nematicidal property, than other secondary metabolites of endophytic fungi. The strength of a compound predicted by their hydrogen bonding complex [17].

Table-2: Hydrogen contacts of the ligand.

Serial number	Compound	Hydrogen contacts
a	Heptelidic acid	SER155,LYS323,GLN327
b	Isocoumarin	GLN324,GLN186,VAL180
c	Phomadecalin E	GLU335,LEU338,ASN331
d	Phomenone	LYS323,ASN68,PRO187,TYR154
e	Peramine	GLY156,TYR154,GLN327,TYR203

Molecular docking studies of marine derived fungal metabolites Hsp90a cancer protein proves their ability to be used as drug leads for cancer treatment [18] from this investigation it was observed that fungal endophytic secondary metabolites such as Heptelidic acid, Isocoumarin, Phomadecalin E, Phomenone, Peramine were more potent ASP-1 inhibitor through comparative docking studies which has lots of promise to develop is ASP-1 inhibitor.

Conclusion

To conclude that the compounds which show better binding features with ASP-1 protein can be explored further using invitro studies, thus these compounds can be effectively used as antinematicidal treatment which is predicted on the basis of docking score .The insights gained in the present work can be further used in experimental studies for designing antinematicidal drug with novel target better mechanism.

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Corresponding Author:

Kalyanaraman Rajagopal*,

Email: *ammaaappa@gmail.com*