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Research Article

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Screening of *Annona muricata* for *E. coli* enoyl acyl carrier protein reductase inhibitors by molecular docking

Santhoshkumar Muthu and Brindha Durairaj*

Department of Biochemistry, PSG College of Arts and College, Coimbatore, Tamilnadu, India

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ABSTRACT

Chemical compounds isolated from plant sources are most common natural products that exhibit a broad spectrum of antibacterial activity. In order to decipher antibacterial activity of A.nuricata compounds and growth inhibitors, molecular docking work was carried out to identify active compounds against NADH-dependent E.coli enoyl-ACP-reductase which is involved in mycolicacid biosynthesis. Annona muricata leaves are traditionally used for insecticidal and antimicrobial applications. The target enzyme was downloaded from the protein data bank. PUBCHEM provided the 3D structures of A.muricata compounds namely coreximine, annonamuricatin B, annonamuricin A,B, annonacin, corrossolone, murisolin, syepharine, and coclaurine. Docking results also revealed that coreximine, syepharine, and coclaurine were found to interact with enzyme and inhibit the active site. This study provides important clues to understanding antibacterial compounds of A.muricata and the results obtained will be useful in predicting natural products.

Keywords: coreximine, enoyl-ACP reductase, *Escherichia coli*, Annonaceae, AutoDock.

INTRODUCTION

The well known drawback of the antibacterial drug therapy is due to the multi drug resistance (MDR) developed by the bacterial strains towards current antibiotics. The failure is associated with the mutations in strains and non compliance in the antibiotic therapy [1]. Hence, the researchers are focusing on enzymes and receptors which are promising targets for the development of effective alternative antibiotics. NADH dependant enoyl acyl carrier protein reductase (FabI) is a key enzyme involved in the last step of each fatty acid elongation reaction. These fatty acids are essential precursors for mycolic acid biosynthesis [2]. Hence, FabI has drawn the attention of researchers to develop FabI-inhibitors as it is the valid target for antibacterial therapy. Many plant derived compounds have been identified to possess antibacterial effect since they inhibit the growth and replication of pathogenic bacterial strains that cause human illness, such as Escherichia, Gingiva, Pseudomonas, Staphylococcus, and Canadida genera [3]. Flavanoids and other phytochemicals act as potential natural sources of antimicrobial drugs due to their interaction with the enoyl acyl carrier protein reductase (FabI) [4]. Therefore, bioactive compounds will be the best choice for identifying novel types of effective antimicrobial and improving the shelf life and safety of foods.

Annona muricata L. known as guanabana and soursop is a member Annonaceae family. It is included in custard-apple family due to a custard-like texture of its fruit. It is tree with a height of 5–8 m and roundish canopy [5-6]. This popular fruit tree are widely cultivated in several tropical countries and traditionally consumed for an array of ailments. Graviola leaves are used as a poultice or an infusion externally for skin complaints in children, and for coughs and rheumatism [7]. Previous reports have demonstrated a significant cytotoxicity of *A. muricata* leaves

against various cancers without affecting the normal cells [8]. Ethanolic extract of *A. muricata* leaves was proven to exhibit apoptosis inducing potential against myelogenous leukemic K562 cells [9-10]. Constituents isolated from A. muricata leaves, namely annonaceous acetogenin alkaloids and essential oils, annonaceous acetogenins are strongly implied to be responsible for the promising anticancer effect. The aqueous extract possesses antibacterial effect against *S. aureus and V. cholera* [11]. People are using the *Annona muricata* leaves for insecticidal and antimicrobial applications. However, scientific reports available to validate its traditional uses are lacking. Hence, the principle objective of this study was to screen bioactive compounds of *A.muricata* for their antibacterial activity through molecular docking with NADH dependant enoyl acyl carrier protein reductase (FabI) of *Escherichia coli*.

EXPERIMENTAL SECTION

Ligand and protein preparation

All the ligands were downloaded from chemical databases like pubchem compound or chemspider. The downloaded ligands were converted to pdb format. The crystal structure of the *E.coli* enoyl acyl reductase (1QG6) protein was downloaded from protein data bank. AutoDock uses an adapted AMBER force field and so the atoms of the protein and the ligands have to be set up in accordance with this. The missing hydrogen atoms were added for the ligand and protein. A torsion search was made to the ligand and the default number of torsion is set for each ligand. Then the ligand was saved as *pdbqt* file format for further analysis. Similarly for proteins the heteroatom was removed and additional chains were deleted to get a monomer [12].

Docking procedure

Docking calculations were performed using AutoDock software (version 4.2). Desired compounds were docked into the active site of *E.coli* enoyl-ACP-reductase. In order to assign the perfect grid of each ligand, grid box values were obtained from trial and error and previous studies. The implementing Lamarckian Genetic Algorithm (LGA), considered as one of the best docking methods available in AutoDock, was adopted to perform the molecular docking studies. The parameters for LGA were defined as follows: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; and mutation and crossover rates of 0.02 and 0.8, respectively. Both Autogrid and AutoDock computations were performed on Cygwin and ten independent docking runs were performed for each compound. The docked conformations of each ligand were ranked into clusters based on the binding energy and the top ranked conformations were visually analyzed. Hydrogen bonding and hydrophobic interactions between docked potent agents and macromolecule were analyzed using AutoDock Tools (version1.50) [13-14]. The proper coordinates were set so that the grid can be specific to the binding site of the enzyme. The grids file was generated and saved in *gpf* format with default setting [15]**.**

RESULTS AND DISCUSSION

E.coli enzyme ACP reductase (FabI) is the one that catalyses the synthesis of type 2 fatty acid which are precursors for mycolic acid biosynthesis. This enzyme is a promising target for identification of antibacterial drugs. Hence, FabI enzyme was particularly selected for docking studies with *Annona muricata* compounds. Enzyme was downloaded from PDB (PDB ID-IQG6). It was co-crystallized with triclosan and a co-factor NAD. Triclosan is an active antibiotic drug molecule and well known inhibitor of FabI. Triclosan forms H bond interactions with amino acids Tyr156 with the help of NAD (Figure 1). The two benzene rings of the triclosan also were found to form close contact through hydrophobic interaction with Ile200 and Met 159. Above mentioned interactions were essential for a drug molecule to inhibit the enzyme. For docking studies, the grid was formed surrounding the Tyr156 with inclusion of essential groups from NAD that creates H bond interaction (Fig 2). The standard triclosan could form H bond interaction with Tyr156 and NAD. The OH group of triclosan forms the H bond interaction with docking score -7.82 kcal/mol.

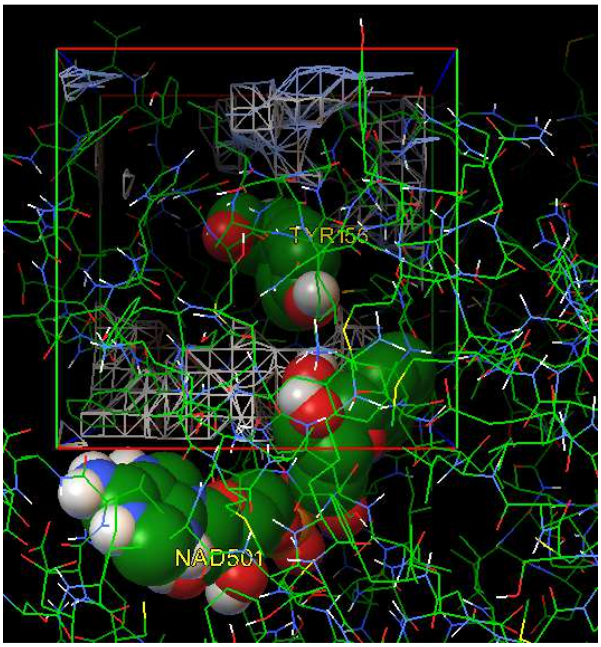

Figure 1: Grid box constructed around the H-Bond interaction forming amino acid (TYR156) along with the co-factor (NAD)

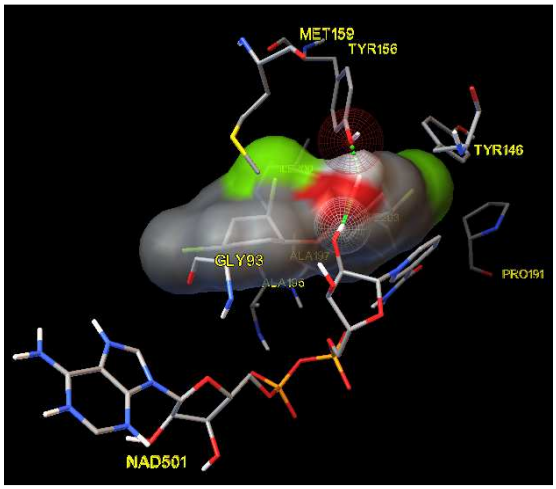

Fig2: Triclosan, H-Bond interaction with Close contact amino acids

Nine compounds of *Annona muricata* were subjected to *in silico* docking against *E.coli* enzyme ACP reductase. Annonamuricatin B, Annonamuricin A and B, Annonacin, Corrossolone and Murisolin could not access the ligand binding pocket in the target. The docking scores have been summarized in Table1. This is due to the large size of molecule that could not be accommodated in the binding pocket of the enzyme. Coclaurine was able to form H-bond interaction with Tyr156 of the target through NAD as in the case of Triclosan. The interaction was seen between OH group of the ligand and H-bond (Figure 3). The oxygen atom of -OH served as an accessor and the H-atom served as a donor. Docking score was found to be - 6.65 Kcal/mol. The coreximine was found to form H-bond interaction with NAD and Gly199 of target enzyme, for which docking scores were recorded as -7.14 Kcal/mol (Figure 4). Syepharine also was observed to exhibit better binding rate with docking score -7.57 Kcal/mol. But it forms H-bond interaction with NAD alone (Figure 5). The selected and optimized lead compounds upon docking studies revealed that there are considerable interactions between ligands and amino acids of the target enzyme.

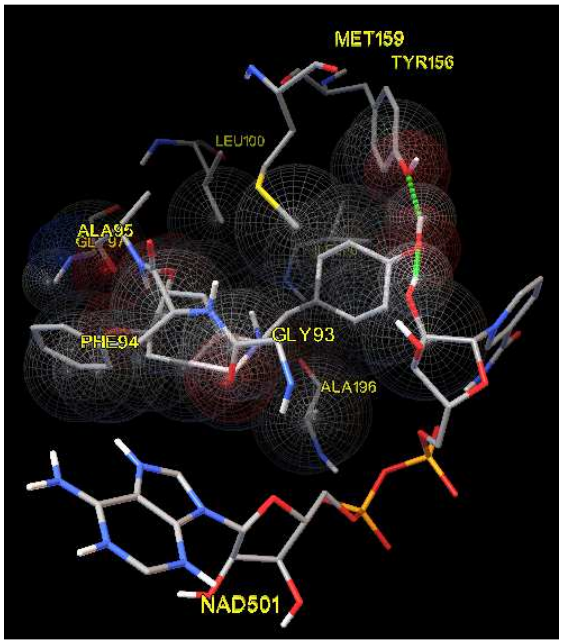

Fig3: Coclaurine, H-Bond interaction with Close contact amino acids

It is reported that FabI usually interacts with Gly93, Gly199, Met103, Met159, Tyr156, Leu195, Ala196, Ile 200 through NAD [16]. In the present study, the hydrogen bonding network was significantly noticed between these optimized ligands (syepharine, coreximine and coclaurine) and the amino acids (Tyr156, Met159, Ile200, Gly199). The results obtained are in agreement with the previous reports [17]. Hence, it is suggested that syepharine, coreximine and coclaurine are the promising lead compounds that can inhibit the ACP reductase (FabI) thereby showing antibacterial efficacy against *E.coli*.

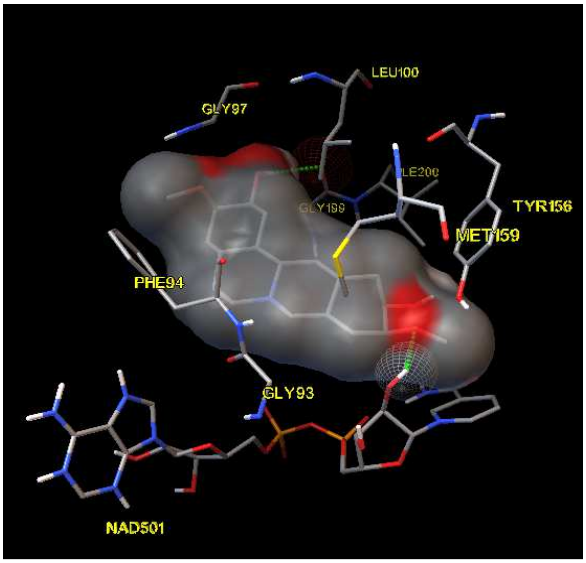

Fig4: Coreximine, H-Bond interaction with Close contact amino acids

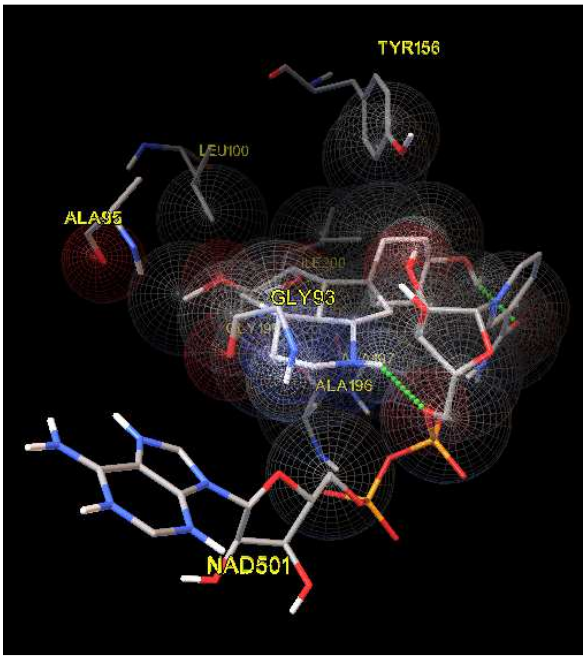

Fig5: Syepharine, H-Bond interaction with Close contact amino acids

Table1: Data of *in silico* Docking Scores

S. No	Ligands	Docking Score Kcal/mol	H-Bond Interaction		
			Y156	NAD	Misc.
1	Triclosan	-7.82	Tyr156	✓	-
2	Annomuricatin B	-	-	-	-
3	Annomuricin A	-	-	-	-
4	Annomuricin B	-	-	-	-
5	Annonacin	-	-	-	-
6	Coclaurine	-6.65	Tyr156	✓	-
7	Coreximine	-7.14	-	✓	Gly199
8	Corrossolone	-	-	-	-
9	Murisolin	-	-	-	-
10	Syepharine	-7.57	-	✓	-

CONCLUSION

The present *in silico* research focused on the design of *E. coli* enoyl ACP reductase inhibitors from *Annona muricata* active compounds. The activity profile of the designed compounds indicated that there existed a significant correlation with the computational data. In conclusion, the highest binding affinity was possessed by syepharine, coreximine and coclaurine with binding energy -7.57, -7.14 and -6.65kcal/mol respectively. The findings reveal that *Annona murocata* may provide excellent candidates for antibacterial drug discovery.

REFERENCES

[1] J Davies; D Davies, *Microbiol Mol Biol Rev*., **2010**, 74(3), 417–433.
[2] RJ Heath; CO Rock, *J Biol Chem.*, **1995**, 3, 270 (44),26538-42.
[3] AL Shaza Anwar; AF Frdoos Mohammad, *J Microbiol*., **2014**, 7(7), e11370.
[4] J Daniel Dubreuil, *Toxins* (Basel)., 2013, 5(11), **2009**–2041.
[5] SO Adewole; EA Caxton-Martins, *Afr J Biomed Res.*, **2006**, 9(3),173–187.
[6] SO Adewole; JA Ojewole, *Afr J Tra Compl Alter Med.*, **2009**, 6(1),30, 223-233.
[7] E Osorio; GJ Arango; N Jimenez; F Alzate; G Ruiz; D Gutierrez; MA Paco; A Gimenez; S Robledo, J *Ethnopharmacol*., **2007**, 111 (3), 630–635.
[8] VC George; DN Kumar; V Rajkumar; P Suresh; R Ashok, *Asian Pac J Cancer P.*, **2012**, 13(2),699–704.

[9] AU Ezirim; VI Okachi; AB James; OA Adebeshi; S Ogunnowo; OB Odeghe, *Indian J Drugs Dis*., **2013**, 2(3), 142–151.
[10] L Zeng;FE Wu; NH Oberlies; McLaughlin JL; Sastrodihadjo S, *J Nat Prod* **1996**, 59(11):1035–1042.
[11] GH Viera; JA Mourão; AM Angelo; RA Costa; RH Vieira, R*ev Inst Med Trop Sao P*aulo., **2010**, 52(3),129-32.
[12] H Bergler; S Fuchsbichler; G Högenauer; F Turnowsky , *Eur J Biochem.*, **1996**, 242(3), 689-94.
[13] GM Morris ; R Huey ; W Lindstrom ; MF Sanner ; RK Belew ; DS Goodsell ; AJ Olson , *J Comput Chem*., **2009**, 30, 2785.
[14] D Karthik; P Majumder; S Palanisamy; K Khairunnisa; V Venugopal, *Bioinformation*., **2014**, 10(9), 580–585.
[15] PN Andrew ; KC Paul ; AK Jean-Pierre ; JK David ; PS Carlos , *J Cheminform* ., **2011**, 3, 12-19.
[16] CBlue E; TJ Mitchell, *Journal of Infectious Diseases*., **2003**, 71, 4405-4413.
[17] YM Zhang; CO Rock, *J Biol Chem*., **2004**, 279, 30994–1001.