Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

STRUCTURAL ELUCIDATION AND MOLECULAR DOCKING OF NOVEL COMPOUNDS FROM *ANNONAMURICATA* FRUIT REVEAL ANTICANCER POTENTIAL AGAINST PI3K

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ABSTRACT

Cancer is the major public health burden in both developed and developing countries. Several synthetic agents are used to cure the disease but they have theirtoxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents which are safe. Traditionally, drugs were discovered by developing synthetic compounds which is a time consuming multi-step processes against a battery of *in vivo*biological screens and further investigating the promising candidates for their pharmacokinetic properties, metabolism and potential toxicity. Sophisticated *Insilico* approaches has given a tremendous opportunity to pharmaceutical companies to identify new potential drug targets which in turn affect the success and time of performing clinical trials for discovering new drug targets. The aim of this research is to explore the antioxidant and anticancer activities of *Annonamuricata*. In present study using TLC-bio -autography and *insilico* screening, structure based molecular dockingoffour compoundsderived from chloroform extract of fruit of *Annonamuricata* was performed. In the present study, software like Autodock 1.5.6, Marvin view and Discovery studio 4.1visualizer were used. The compounds under investigation were Phytosphingosine, Dihydroergocoronine, Cetylpyridinium, and Dihydrospingosine. These four compounds are obtained from HR-LCMS analysis and these fourcompounds were docked against the5itd, it which is the PDB ID for PI3Kprotein derived from cancer pathway. The binding energies of these compounds; viz. Phytosphingosine, Dihydroergocoronine, Cetylpyridium, and Dihydrospingosine are -4.82kcal/mol, -7.93kcal/mol, -5.24kcal/mol,-3.97 kcal/mol respectively. This,*Insilico* approach reveals phytochemicals from *Annonamuricata* extract shows the potential to be an anticancer agent.

KEYWORDS: ***Antioxidants, Cancer, TLC- bio-autography,Annonamuricata,In-Silico, Molecular docking.***

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Received on: 19-07-2017
Revised and Accepted on: 09-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b445-451

INTRODUCTION

Cancer is a one of the most dreadful diseases, which is spreading with high frequency. Studies evaluated that,each year more than 10million new cases of cancer are reported around the world, with more than 6 million deaths in the year 2000[1].Cancer is the second reason for deaths and have the highest burden of diseases in 21st century. As indicated by the WHO; world health statistical report 2012,the yearly deaths will increase from 7.6 million in 2000 to 13 million in 2030[2]. Several researches show that ingestion of natural extracts containing antioxidants in their composition, particularly polyphenolic compounds, are connected with lower rate of coronary illness, cancers and diabetes[3].Recently, more than 3000 plantsfrom different partsof the world have been accounted foranticancer properties. The prevalence of products derived from plants for the treatment of cancer is from 10% to 40% and this rate now came up to 50% in Asiatic patients. Around 60% of the drugs for cancer treatment have been isolated from natural product.[4]Antioxidants are a kind of complex compounds found in our diet that act as a defensive shield for our body against certain disastrous diseasessuch as cardiovascular ailments, joint inflammation cataract and premature ageing along with several chronic diseases. Plants contain certain chemicals such as carotenoids, flavonoids, biflavonoids, phenols, and phytosterols etc. that have antioxidative properties. Since reactive oxygen radicals play a vital part in carcinogenesis and other human disease states, antioxidants present in plants have received significant consideration as cancerchemo preventive agents[5].One of the important herbal plant *Annonamuricata* L. which is commonly known as sour sop attracts greater attention because of it's high medicina value; both in herbal folklore practises,it has been found to be a promisingantitumor agent in numerous *invitro* studies[6].Molecular docking, structural based screening and post-screening are emerging trends in molecular biology and computer-assisted drug design. The objective of molecular docking is to determine the binding interactions between two molecules - either protein to protein or protein to ligand. Once a compound is docked, Scoring estimates the chemical interactions such a binding strength and energy state, between the ligand and protein to help with positioning the adequacy of the compound being scored. From these approaches, compounds that directly interact with target proteins can be identified[7].Identification of dynamic standard which is known as biologically active maker compound requires standardization;using appropriate chemical procedures such as chromatographic and spectral studies.Several screening assays are available for the screening of potential anti-oxidantsin which Thin layer chromatography (TLC) bio-autography assay is the best strategy. Comparing with other methods, TLC bio-autography is quick, easy,convenient, simple and efficient method for identification of active components from a complicated plant extract. So for the screening of antioxidants, the TLC bio- autography assay is the great choice. Several TLC techniques are developed and utilized for qualitative and quantitative analysis of antioxidants but these methods use stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as derivatizing agent[8].

MATERIALS AND METHODS

Chemicals

In present study, chemicals used DPPH, Solvents such as Ethanol, Chloroform, Toluene, Methanol, were purchased from Himedia.TLC silica gel 60 F 254 plates were purchased from (Merck,KGaA, Germany) and different data bases like PUBCHEM, DRUGBANK, PDB(protein data bank)and softwares such as Autodock 1.5.6, Discovery Studio Visualizer 4.1 and Marvin view.PDB is the single universal data bank of biological macro molecules as it contains structural evidence of macromolecules determined by spectroscopy.

Plant material

Annonamuricata is native to the tropical region and in India it is found in Maharashtra, Andhra Pradesh,Tamilnadu,and Karnataka states.20gm of fruit powder was extracted using soxhlet apparatus. The extract wasfiltered, concentrated by boiling, dried and stored in refrigerator until further use[9].

Thin Layer Chromatography:DPPH autography

The extract was chromatographed using TLC silica plate (Merck F254) with Toluene, Chloroform, and Methanolin the ratio 4.5:5:0.5 with their appropriate ratios as mobile phase. Developed plates were dried, sprayed with DPPH solution and observed for bright colour yellow bands on purple background for conformation as antioxidant compound.

Docking

For docking of two molecules, either protein to protein or protein to ligand Autodock Tools version 1.5.6was used.

Protein–Ligand docking

Preparation of ligand

The compounds were obtained from LCMS data, these compounds were found from Pubchem and their structures were derived in SDF format. The compoundswereprocessed using Marvin view application, then the structure were cleaned, 2D and 3D the structurewas thensaved in Mol2 format, Later on, ligand was opened in Autodock tool set the number of torsions, and the ligand in PDBQT format was saved.

Preparation of protein

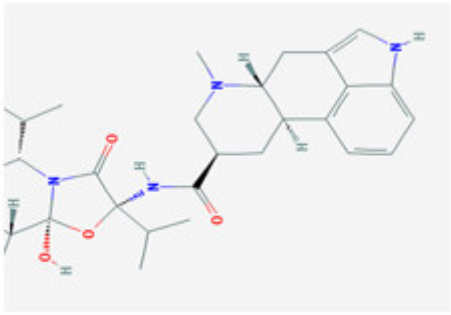
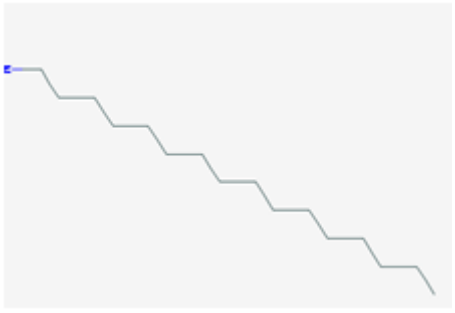
The protein structure is obtained from PDB, then the protein is opened in discovery studio visualisar, the water molecules and other ligand group are removed followed by addition ofhydrogen which ispolar thenthe file was saved in PDBQT format. Ligplot of the protein is obtained from PDBSUM to know the x, y,z, co-ordinates.

Grid preparation

In grid preparation, the target protein PDB structure was opened in Autodock, instantly the PDB file will be converted into PDBQT file inside the same path, later on add atoms directly like A C H Cl Br I etc, to set the map. Then arrange the x, y, z co-ordinates as 60 x60x60 and save the file as protein GPF. Default optimization

parameters were done with Lamarkian Genetic Algorithm. Autodock tools generated sixty possible conformations,i.e. sixty runs for each docking. After grid preparation,dock the ligand was dockedagainst the target protein. After completion of docking most suitable conformations were selected based on lowest binding energy.

Mass spectroscopic data of Compounds

Structure	m/z	Formula
	318.29	$C_{18}H_{39}NO_3$
Figure 1 ***Phytosphingosine***		
	538.29	$C_{31}H_{41}N_5O_5$
Figure 2 ***Dihydroergocoronine***		
	304.29	$C_{21}H_{38}N$
Figure 3 ***Cetylpyridinium***		
	302.3	$C_{18}H_{39}NO$
Figure 4 ***Dihydrospingosine***		

RESULTS AND DISCUSSION

Annonamuricata leaves extract was known for broad use in the medical field both traditionally and pharmaceutically. so there is greater possibility of it's use a santi-inflammatory, anti-allergic, antibacterial, and antiviral, antioxidant and anticancer agent[10].Previous reports have demonstrated a significant cytotoxicity of *Annonamuricata* leaves against various cancers without affecting the normal cells[11].In the present study,*Insilco* approach is used for compounds obtained by HR-LCMS data from chloroform extract of *Annonamuricata* fruit. Four compounds Phytosphingosine (Fig1), Dihydroergocoronine (Fig. 2), Cetylpyridinium (Fig. 3) and Dihydrospingosine (Fig. 4)are identified as ligands in PDBQT format and docked with target protein 5ITD derived from PI3K cancer inhibitor at the active site. Their binding energies are predicted to be -4.82 kcal/mol

(Fig. 7), for Phytospingosine,-7.93kcal/mol (Fig. 9) for Dihydroergocoronine,5.24 kcal/mol (Fig. 11) for Cetylpyridiniumand -3.97kcal/mol (Fig. 13) for Dihydrospingosine but Cetylpyridinium does not show any hydrogen bond with protein. Protein and ligand complex models were generated after successful docking obtained based on the parameters such as hydrogen bond interactions, electrostatic interactions, binding energy, RMSD of active site residues and orientation of the docked compound within the active siteas shown in table 1[12].Thin layer chromatography DPPH bio- autography from *Annonamuricata* chloroform extract also shows (Fig.5) presence of antioxidant compounds.Purple colour of DPPH reagent was bleached which indicated positive antioxidant activity[13].

Figure 5
DPPH Bio-Autography by TLC

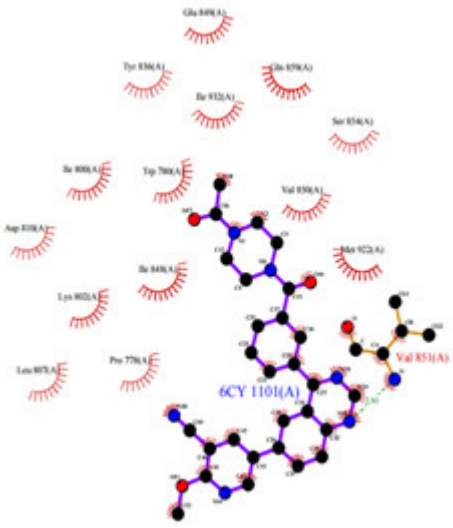


Figure 6
Lig Plot For Target Protien 5ITD

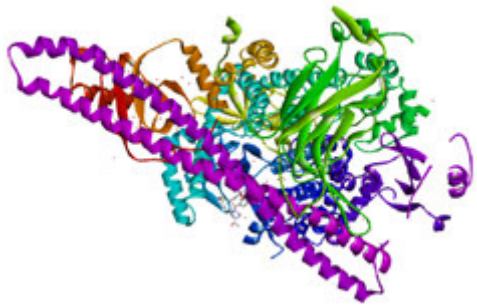


Figure 7
3D structure of phytospingosine and 5itd protien complex

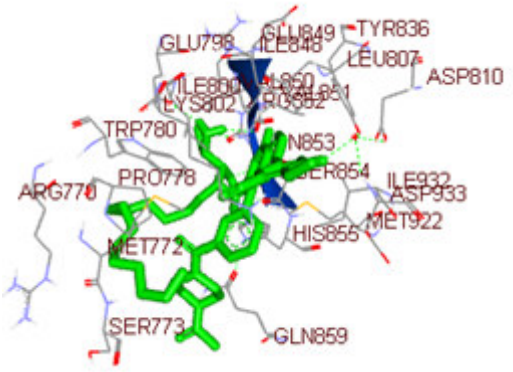


Figure 8
Amino Acid Bind To The Complex

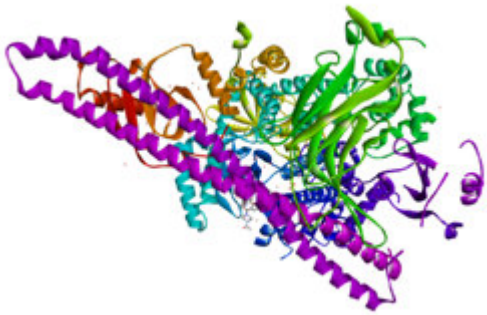


Figure 9
3D Structure Of Dihydroergocoronine And 5itd Protein

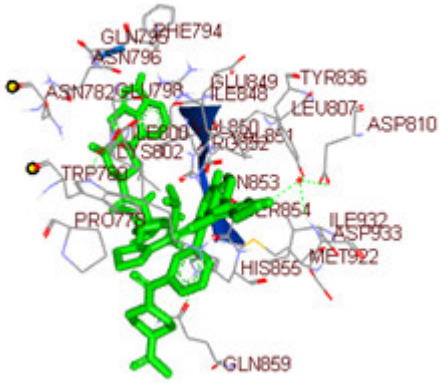


Figure 10
Amino Acids Bind To Complex.

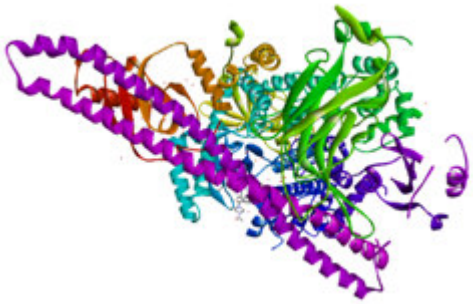


Figure 11
3D structure Of Cetylpyridinium-5itd Protien Complex

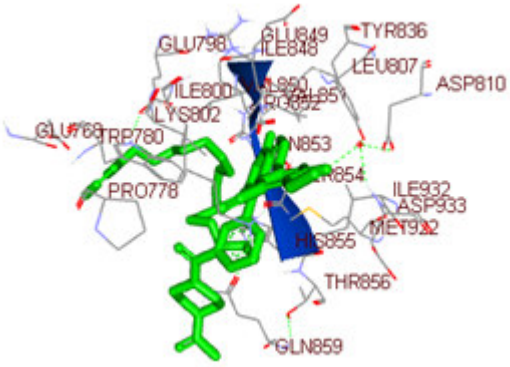


Figure 12
Amino Acids Bind to The Complex

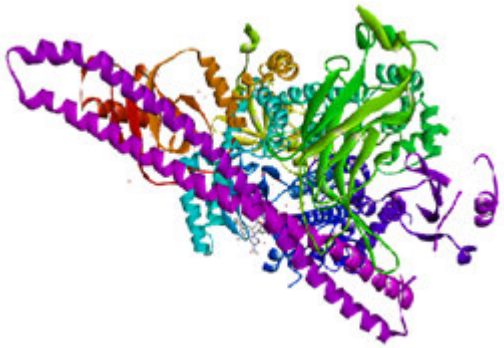


Figure 13
3D Structure Of Dihydrosphingosine and 5itd Protein

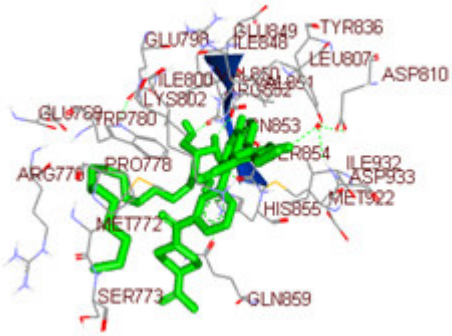


Figure 14
Amino Acid Bind To The Complex.

Table 1
Energy table of protein 5ITD with the compounds

Sr. no.	Ligand	Run No	Interacting residue	Interacting atoms(Aminoacid…Ligand)	Hbond formed	Bindig Energy	Electrostatic energy
1	Phytospingosine	56	ASN853 VAL851 SER854 SER854	HN……O3 H31……O H30……O HN…….O1	4	-4.82	-0.08
2	Dihydroergocoronine	21	6CY1101	6CY1101	1	-7.93	-0.06
3	Cetylpyridinium	47	-	-	-	-5.24	-1.48
4	Dihydrospingosine	22	ASN853 6CY1101	HN H28	2	-3.97	-0.05

CONCLUSION

The objective of the study was to determine the *in vitro* anti-oxidants and *in silico* anticancer potential of chloroform extract of *Annonamuricata* fruit. The compounds obtained using the HR-LCMS analysis. Thin layer chromatography (TLC) bio-autography reveals the presence of antioxidants as yellow band on the purple background on TLC plate, when those were sprayed with DPPH. The *Insilico* approach for these four compounds points out that they possess anticancer activity. The four compounds successfully docked with the protein 5ITD. As these compounds i.e.Phytosphingosine, Dihydroergocoronine, Cetylpyridinium and Dihydrospingosine showed the binding energy as,-4.82kal/mol, -7.93kal/mol, -5.24kcal/mol and - 3.97kcal/mol respectively while Cetylperidinium showed binding energy but lacked hydrogen bonds. Compounds identified in this study show a lot of potential for further in vivo studies leading to alternative use for cancer treatment.

FUNDING ACKNOWLEDGEMENT

Author Sandhyarani is thankful to University Grants Commission (UGC), New Delhi, India for financial support in the form of Rajiv Gandhi National Fellowship (F1-17.1/2016-17-SC-AND-6000/(SA-III/Website) and IIT, Mumbai for providing HR-LCMS facilities.

CONFLICT OF INTEREST

Conflict of interest declared none.

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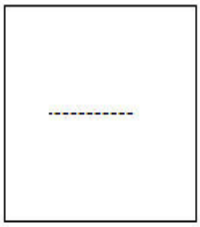

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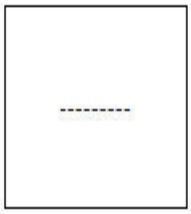

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We sincerely thank the above reviewers for peer reviewing the manuscript