



## 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 their toxicity 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 *In silico* 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 *in silico* screening, structure based molecular docking of four compounds derived from chloroform extract of fruit of *Annonamuricata* was performed. In the present study, software like Autodock 1.5.6, Marvin view and Discovery studio 4.1 visualizer were used. The compounds under investigation were Phytosphingosine, Dihydroergocoronine, Cetylpyridinium, and Dihydrospingosine. These four compounds are obtained from HR-LCMS analysis and these four compounds were docked against the 5itd, it which is the PDB ID for PI3K protein derived from cancer pathway. The binding energies of these compounds; viz. Phytosphingosine, Dihydroergocoronine, Cetylpyridium, and Dihydrospingosine are -4.82 kcal/mol, -7.93 kcal/mol, -5.24 kcal/mol, -3.97 kcal/mol respectively. This, *In silico* 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>



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## INTRODUCTION

Cancer is one of the most dreadful diseases, which is spreading with high frequency. Studies evaluated that each year more than 10 million new cases of cancer are reported around the world, with more than 6 million deaths in the year 2000<sup>1</sup>. Cancer is the second reason for deaths and has the highest burden of diseases in the 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<sup>2</sup>. Several researches show that ingestion of natural extracts containing antioxidants in their composition, particularly polyphenolic compounds, are connected with a lower rate of coronary illness, cancers and diabetes<sup>3</sup>. Recently, more than 3000 plants from different parts of the world have been accounted for anticancer 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 products<sup>4</sup>. Antioxidants are a kind of complex compounds found in our diet that act as a defensive shield for our body against certain disastrous diseases such 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 cancer chemo preventive agents<sup>5</sup>. One of the important herbal plants, *Annonamuricata* L. which is commonly known as sour sop, attracts greater attention because of its high medicinal value; both in herbal folkloric practices, it has been found to be a promising antitumor agent in numerous *in vitro* studies<sup>6</sup>. 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 as 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<sup>7</sup>. Identification of dynamic standard which is known as biologically active marker compound requires standardization; using appropriate chemical procedures such as chromatographic and spectral studies. Several screening assays are available for the screening of potential anti-oxidants in 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<sup>8</sup>.

## 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 macromolecules 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. 20 gm of fruit powder was extracted using Soxhlet apparatus. The extract was filtered, concentrated by boiling, dried and stored in refrigerator until further use<sup>9</sup>.

### Thin Layer Chromatography: DPPH autography

The extract was chromatographed using TLC silica plate (Merck F254) with Toluene, Chloroform, and Methanol in 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 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 1.5.6 was 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 compounds were reprocessed using Marvin view application, then the structure were cleaned, 2D and 3D the structure was then saved 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 Visualizer, the water molecules and other ligand group are removed followed by addition of hydrogen which is polar then the file was saved in PDBQT format. Ligplot of the protein is obtained from PDBSUM to know the x, y, z, co-ordinates.

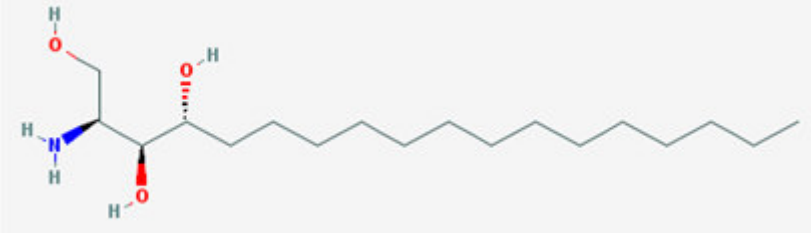
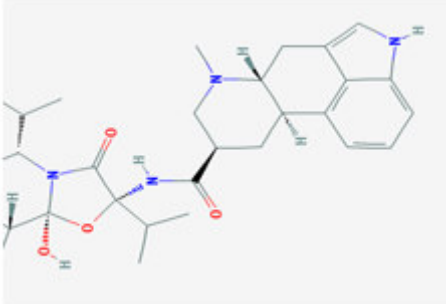
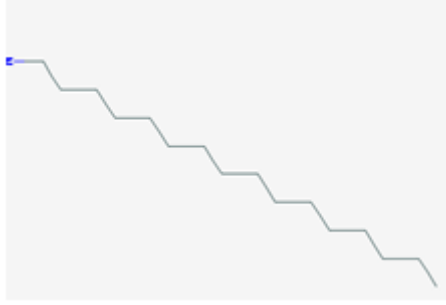

#### Grid preparation

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 x 60 x 60 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 docked against 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
 <p>Chemical structure of Phytosphingosine, showing a long hydrocarbon chain, a primary amine group, and a primary alcohol group.</p>	318.29	C <sub>18</sub> H <sub>39</sub> NO <sub>3</sub>
<p><b>Figure 1</b> <b>Phytosphingosine</b></p>		
 <p>Chemical structure of Dihydroergocoronine, a complex polycyclic alkaloid with multiple nitrogen atoms and a long side chain.</p>	538.29	C <sub>31</sub> H <sub>41</sub> N <sub>5</sub> O <sub>5</sub>
<p><b>Figure 2</b> <b>Dihydroergocoronine</b></p>		
 <p>Chemical structure of Cetylpyridinium, consisting of a long hydrocarbon chain attached to a pyridinium ring.</p>	304.29	C <sub>21</sub> H <sub>38</sub> N
<p><b>Figure 3</b> <b>Cetylpyridinium</b></p>		
 <p>Chemical structure of Dihydrospingosine, showing a long hydrocarbon chain, a secondary amine group, and a primary alcohol group.</p>	302.3	C <sub>18</sub> H <sub>39</sub> NO
<p><b>Figure 4</b> <b>Dihydrospingosine</b></p>		

## 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 its use as a anti-inflammatory, anti-allergic, antibacterial, and antiviral, antioxidant and anticancer agent<sup>10</sup>. Previous reports have demonstrated a significant cytotoxicity of *Annonamuricata* leaves against various cancers without

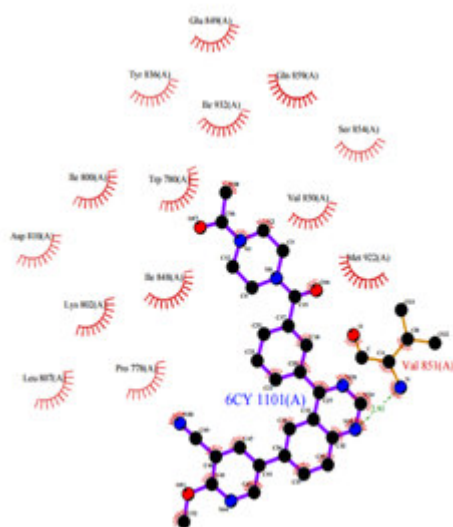
affecting the normal cells<sup>11</sup>. In the present study, *In silico* approach is used for compounds obtained by HR-LCMS data from chloroform extract of *Annonamuricata* fruit. Four compounds Phytosphingosine (Fig1), Dihydroergocoronine (Fig. 2), Cetylpyridinium (Fig. 3) and Dihydrospingosine (Fig. 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 Cetylpyridinium and -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 site as shown in table 1<sup>12</sup>. 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<sup>13</sup>.



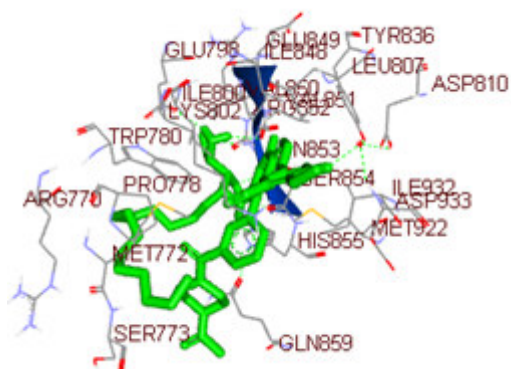
**Figure 5**  
**DPPH Bio-Autography by TLC**



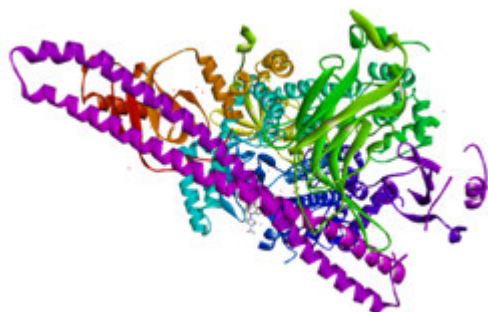
**Figure 6**  
**Lig Plot For Target Protein 5ITD**



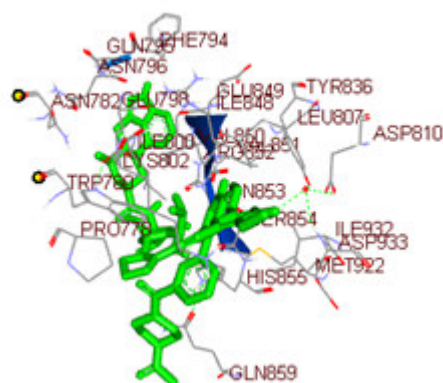
**Figure 7**  
*3D structure of phytospingosine and 5itd protien complex*



**Figure 8**  
*Amino Acid Bind To The Complex*



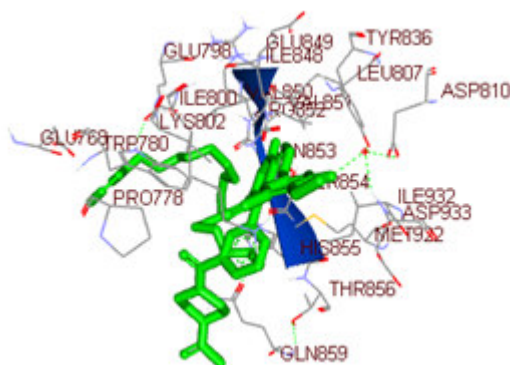
**Figure 9**  
*3D Structure Of Dihydroergocoronine And 5itd Protein*



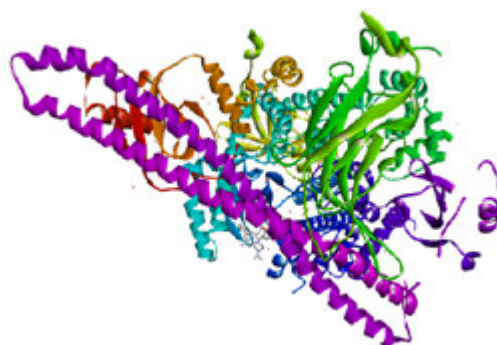
**Figure 10**  
*Amino Acids Bind To Complex.*



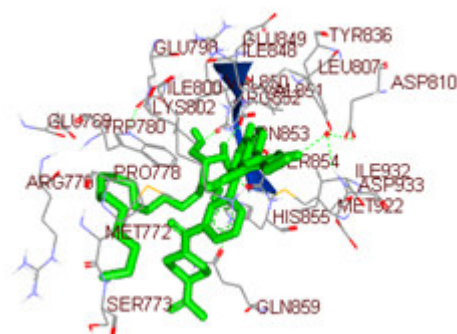
**Figure 11**  
**3D structure Of Cetylpyridinium-5itd Protien Complex**



**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 *In silico* 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. Phytospingosine, Dihydroergocoronine, Cetylpyridinium and Dihydrospingosine showed the binding energy as, -4.82kcal/mol, -7.93kcal/mol, -5.24kcal/mol and -3.97kcal/mol respectively while Cetylperidinium showed binding energy but lacked

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hydrogen bonds. Compounds identified in this study show a lot of potential for further *in vivo* studies leading to alternative use for cancer treatment.

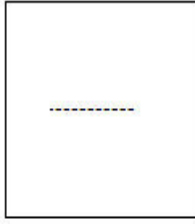
## FUNDING ACKNOWLEDGEMENT

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

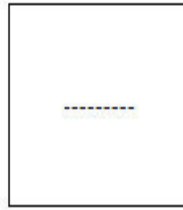
Conflict of interest declared none.

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