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ANTICANCER ACTIVITY OF SECONDARY METABOLITES FROM SOIL GRAM-NEGATIVE BACTERIA PSEUDOMONAS FLUORESCENS (ATCC 135250) AGAINST ACUTE LYMPHOBLASTIC LEUKAEMIA USING MOLECULAR DOCKING

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ABSTRACT

Acute lymphoblastic leukaemia (ALL) is a common malignancy of children of 2-10 years, peak incidence at 4-5 years of age. In India, 60-85% of all leukaemia reported are acute lymphoblastic leukaemia. Currently, acute lymphoblastic leukaemia treatment such as chemotherapy, radiotherapy and stem cell transplantation are at higher risks in children. Therefore, soil microbial secondary metabolites have been considered one of the powerful resources for drugs used to treat acute lymphoblastic leukaemia. In this study, the binding interaction between CD19 (blast cell lines protein) and Ligand (secondary metabolites) through molecular docking approach were studied. The protein CD19 (PDB id: 6AL5) was retrieved from Protein Data Bank (PDB) and the Ligand secondary metabolites were drawn from PubChem database. After that, the protein and Ligand structures were optimized. Lastly, the blast cell line protein was docked with Ligands such as chitinase, pyrrolnitrin, pyoluteorin, salicylic acid, 2, 4-Diacetyl phloroglucinol using Auto Dock. All the Ligand structures were successfully docked with the target protein. The secondary metabolites chitinase, pyrrolnitrin, pyoluteorin, salicylic acid, 2,4-Diacetyl phloroglucinol showed binding energies with CD19 at -6.04 Kcal/mol, -6.01 Kcal/mol, -5.57 Kcal/mol, 5.12 Kcal/mol, -4.40 Kcal/mol respectively. The chitinase had the strongest bond with CD19 protein. Hence, Secondary metabolites can be used to treat acute lymphoblastic leukaemia. Therefore, it can further be evaluated in laboratory experiments

Keywords: Acute Lymphoblastic Leukaemia, Secondary Metabolites, Molecular Docking.

INTRODUCTION

Among the various human diseases, cancer has proven to be one of the most uncontrollable diseases to which humans are subjected and more than 100 different types of cancer have been identified till date and their symptoms vary widely [7]. In recent years, acute lymphoblastic leukaemia is considered to be the most common cancer among children [10].

Despite the progress achieved in its treatment for children, some do not respond to standard treatment or experience side effects of chemotherapy and radiation therapy for the rest of their lives and as practical and generally effective drugs or methods of control are not available yet.

This highlights the need of less dangerous treatments. Natural source of medicinal agents plays significant roles in improving human health and wellbeing, including their application in cancer prevention and treatment regimens [2]. Among such natural products soil microbial secondary metabolites have been considered one of the useful compounds in the treatment of cancer. A rich diversity of secondary metabolites produced by pseudomonas fluorescens had been admired. The bacteria are gram-negative, motile rods, that are primarily aerobic,

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to ferment glucose, and chemo-organotrophic and grow at a pH between 4-8, produce a fluorescent pigment (pyocyanin) from which the name pseudomonas fluorescens is derived[12]. Many investigations had been made to evaluate the anticancer activity of pseudomonas fluorescens. Hence, in our study, we selected secondary metabolites of soil gram negative Pseudomonas fluorescens bacteria.

As a matter of fact, that computational approaches, such as structural bioinformatics, molecular docking, pharmacophore modelling are best choice[11]. In this present research, the anti-cancer activity of secondary metabolites (Pseudomonas fluorescens) [Fig: 1] was screened against acute lymphocytic leukaemia cell lines using molecular docking. Molecular docking is used to calculate the binding affinity of ligand molecules, which is important in understanding their metabolic activities. The discovery of the protein target along with its regulator is typically the first approach to find the novel pharmacological compounds.

MATERIALS AND METHODS

Materials

For our present study we used biological databases like PubChem, PDB (Protein Data Bank) and software's like AutoDock, Mgltools, Pymol. The PDB (Protein Data Bank) is the single worldwide archive of structural data of biological macromolecules, established in Brookhaven National Laboratories (BNL) in 1971. PubChem is a public repository for information on chemical substances and their biological activities, launched in 2004 as a component of the Molecular Libraries Roadmap Initiatives of the US National Institutes of Health (NIH). PubChem consists of three inter-linked databases, substance, compound and bioassay. AutoDock is a computerized suite of protein-ligand docking tools and it offers excellent on-screen molecule-building facilities.

Methodology

Prior to the docking studies, the structures of the ligand and the target protein were processed. The starting structure of the target protein (PDB id: 6AL5) [Fig: 2&3] required for docking was retrieved from the protein data bank repository (http://www.rcsb.org). The protein was prepared for docking studies as follows: water and ligand coordinates were deleted from the protein file. The polar hydrogens were added and after determining the Kolman united atom charges, AD4 type atoms were assigned using AutoDock tools.

For ligand preparation, three dimensional (3D) structures of the ligand (secondary metabolites) [Fig: 4, 9, 10, 11&12] were drawn from PubChem repository (https://pubchem.ncbi.nlm.nih.gov) and optimized for docking studies. These optimized structures were used as inputs of the AutoDock tools. Then the partial charges of atoms were calculated using the Gasteiger charges procedure implemented in the AutoDock tools package. Non-polar hydrogens of the compounds were merged and then rotatable bonds were defined. Prepared protein and ligand structures were saved in the PDBQT format for calculating energy grid maps. The molecular docking technique was conducted using the Autodock 4.2 software package, with the implemented empirical free energy function and the Lamarckian genetic algorithm5. Molecular docking calculations were performed through Autodock via blind docking. These molecular docking scores were obtained after running Autogrid and Autodock in the GLG and DLG file format. The scoring function (binding energies and RMSD value) are obtained from the above said two files. Docking allows virtually screening a database compounds and predicting the strongest binders based on their scoring functions. Protein, ligand, docking images were saved from Autodock. The RMSD table snaps were taken using snipping tool.

RESULT

The docking results of secondary metabolites [Fig: 1] including the evaluated free binding energy values of the docked positions, presented in kcal/mol, and the RMSD values, presented in Å expressed in [Table1].

The CD19, CD20, CD10, CD2, CD8, CD5 Proteins are important markers used to identify Acute lymphoblastic leukaemia. We selected CD19 protein as a molecular target to prevent acute lymphoblastic leukaemia. These bioactive compounds are secondary metabolites such as 2,4-Diacetylphloroglucinol, Pyoluteorin, Pyrrolnitrin, Cyclic lipopeptides, Hydrogen cyanide, Pyoverdine, Salicylic acid, Siderophore, Chitinase, Phenazines, Pseudobactin, Based on the availability of 3D structure from database, molecular docking analysis was carried out with 5 bioactive compounds from Pseudomonas Fluorescens such as chitinase, pyrrolnitrin, pyoluteorin, salicylic acid, 2,4-Diacetyl phloroglucinol.

These compounds were further screened against the target acute lymphoblastic leukaemia protein CD19. Among the 5 compounds from Pseudomonas Fluorescens, the compound chitinase showed potential binding energy of -6.04 Kcal/mol with the targeted protein CD19 followed by the pyrrolnitrin, pyoluteorin, salicylic acid, 2,4-Diacetyl phloroglucinol, with the binding energies of -6.01 Kcal/mol, -5.57Kcal/mol, -5.12Kcal/mol, -4.40Kcal/mol, respectively [Table1].

DISCUSSION

The natural bioactive compounds isolated from a variety of microbes are considered to be safe and less toxic compared to synthetic drugs. In order to study the efficacy of these compounds, it is essential to understand its structure and function. The prediction of ligand binding sites is important in drug discovery to facilitate the optimization process. Most desirable therapeutic agents are

usually required in micro quantities but expressing a high degree of purity and specificity.

According to Abdul Khader Sultan Mohideen (2020), the enzyme L-ASP I from Vibirio campbellii were successfully docked with its substrate L-Asn. The binding energy were recorded to be -7.45 Kcal/mol. This in silico approach will ensure stability, safety and efficacy of antileukemic agent in the treatment and management of Acute Lymphoblastic Leukaemia1. In the present study, molecular docking results provided detailed structural insights of secondary metabolites Pseudomonas Fluorescens and CD19 blast cell lines. The binding energies of secondary metabolites with leukemic cell line protein were -6.04Kcal/mol, -6.01Kcal/mol, -5.57Kcal/mol, -5.12Kcal/mol, -4.40Kcal/mol. The stronger binding is confirmed from more negative docking scores. According to the lowest binding score, the chitinase had the strongest binding affinity with protein CD19.

The biological activity of any drug is dependent on the binding energy between protein and ligand. The present investigation has clearly demonstrated that chitinase have stronger binding energy with protein CD 19. Hence, secondary metabolites of Pseudomonas Fluorescens (chitinase, pyrrolnitrin, pyoluteorin, salicylic acid, 2,4-Diacetyl phloroglucinol) can serve efficiently as anti-leukemic agent in the treatment but further experimental and clinical confirmation is needed to prove it as valid drug.

TABEL 1: Molecular docking analysis of bioactive compounds of Pseudomonas Fluorescens against Acute lymphoblastic leukaemia target protein CD19 (PDB id: 6AL5.

S.NO	COMPOUNDS NAME	BINDING ENERGY (Kcal/mol)	RMSD(Å) VALUE
1.	Chitinase	-6.04	0.76
2.	Pyrrolnitrin	-6.01	0.86
3.	Pyoluteorin	-5.57	0.82
4.	Salicylic acid	-5.12	0.41
5.	2,4-Diacetyl phloroglucinol	-4.40	0.94

TABLE 2: Showing binding energy of chitinase with 6al5

Rank	Sub-Rank	RUN	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	8	-6.04	0.00	60.67	RANKING
2	1	1	-5.73	0.00	72.00	RANKING
3	1	5	-5.33	0.00	85.89	RANKING
4	1	9	-5.16	0.00	84.79	RANKING
4	2	10	-5.14	1.96	84.90	RANKING
4	3	7	-4.78	1.09	85.22	RANKING
4	4	3	-4.64	1.37	85.32	RANKING
5	1	4	-4.95	0.00	72.65	RANKING
6	1	2	-4.60	0.00	85.15	RANKING
7	1	6	-3.71	0.00	60.73	RANKING

TABLE 3: Showing binding energy of pyrrolnitrin with 6al5.

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	7	-6.01	0.00	75.37	RANKING
1	2	10	-6.01	0.08	75.34	RANKING
1	3	6	-6.01	0.04	75.36	RANKING
2	1	2	-5.92	0.00	79.38	RANKING
3	1	4	-5.90	0.00	75.19	RANKING
4	1	8	-5.79	0.00	86.15	RANKING
5	1	3	-5.71	0.00	86.72	RANKING
6	1	5	-5.49	0.00	95.41	RANKING
7	1	9	-5.42	0.00	69.14	RANKING
8	1	1	-5.27	0.00	73.74	RANKING

TABLE 4: Showing binding energy Pyoluteorin with 6al5.

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	10	-5.57	0.00	69.98	RANKING
1	2	3	-5.46	1.63	70.20	RANKING
2	1	6	-5.22	0.00	86.87	RANKING
3	1	8	-4.90	0.00	87.39	RANKING
3	2	5	-4.87	0.18	87.36	RANKING
4	1	9	-4.80	0.00	73.03	RANKING
4	2	7	-4.75	0.89	72.97	RANKING
5	1	1	-4.76	0.00	65.64	RANKING
6	1	2	-4.59	0.00	73.20	RANKING
7	1	4	-4.52	0.00	97.80	RANKING

TABLE 5: Showing binding energy of salicylic acid with 6al5

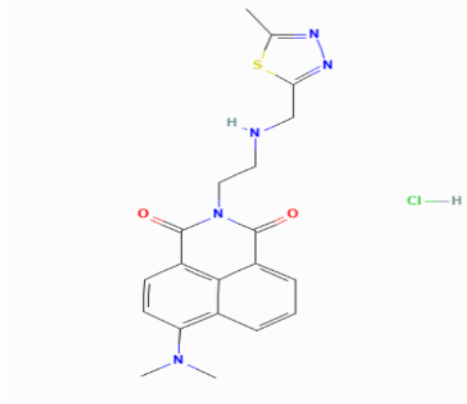
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	4	-5.26	0.00	76.93	RANKING
1	2	9	-5.26	0.86	76.78	RANKING
1	3	8	-5.18	0.87	76.80	RANKING
1	4	10	-5.13	0.78	76.83	RANKING
1	5	2	-5.13	0.71	76.91	RANKING
1	6	6	-4.97	1.24	77.00	RANKING
1	7	7	-4.89	1.15	76.91	RANKING
2	1	1	-5.19	0.00	90.33	RANKING
3	1	5	-4.28	0.00	71.59	RANKING
4	1	3	-3.71	0.00	71.81	RANKING

TABLE 6: Showing binding energy of 2,4-Diacetyl phloroglucinol with 6al5

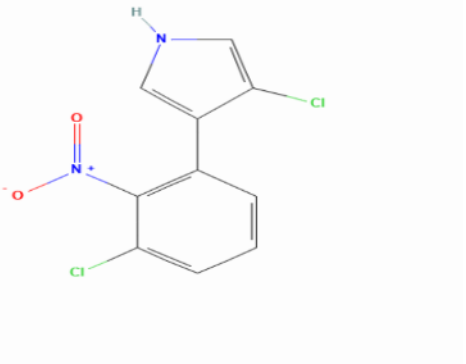
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	7	-4.40	0.00	86.90	RANKING
2	1	3	-4.33	0.00	86.75	RANKING
3	1	9	-4.15	0.00	87.17	RANKING
3	2	8	-4.00	1.84	87.21	RANKING
4	1	5	-3.92	0.00	76.55	RANKING
5	1	1	-3.79	0.00	81.04	RANKING
6	1	4	-3.78	0.00	79.47	RANKING
7	1	10	-3.75	0.00	86.52	RANKING
8	1	6	-3.53	0.00	74.44	RANKING
9	1	2	-3.33	0.00	89.85	RANKING

Fig.1: Secondary metabolites of Pseudomonas Fluorescens

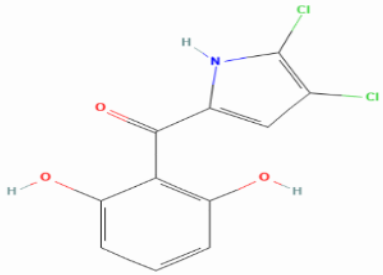
a) Chitinase



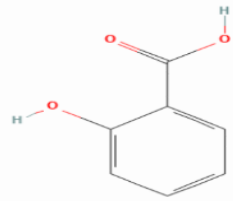
b) Pyrrolnitrin



c) Pyoluteorin



d) Salicylic acid



e) 2,4-Diacetylphloroglucinol

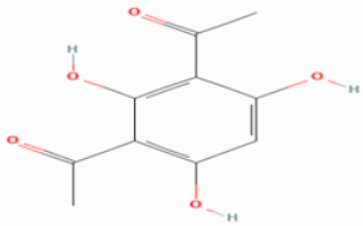


Fig 2: Protein 6AL5 3D structure of Blast cell lines of Acute lymphoblastic leukaemia

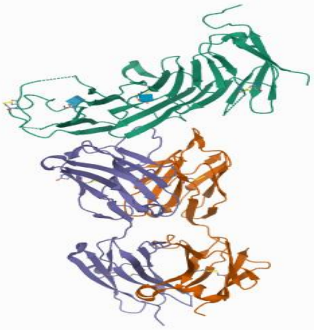


Fig 3: 6AL5 protein of Blast cell lines of Acute lymphoblastic leukaemia

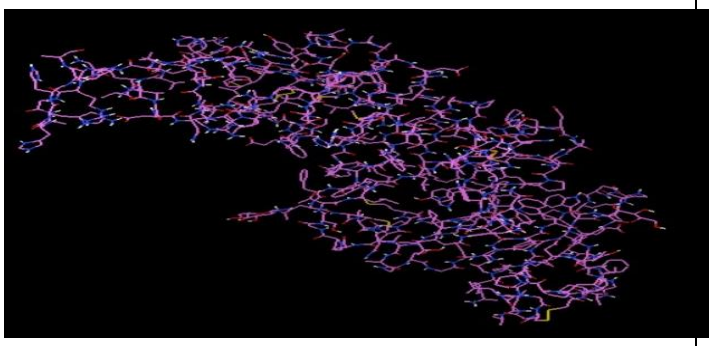


Fig 4: 3D Structure of Chitinase ligand of Pseudomonas Fluorescens

Fig5: Chitinase ligand Interaction with protein CD19

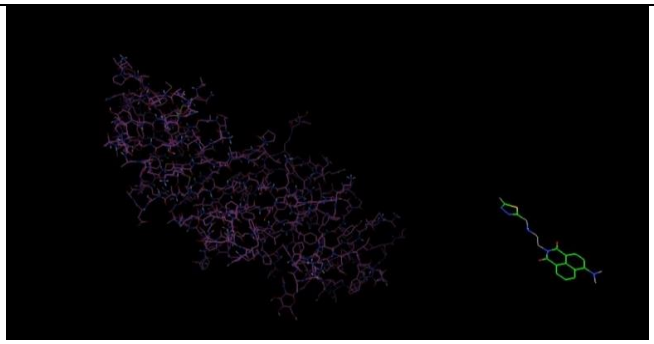


Fig 6: Grid generation of protein CD19

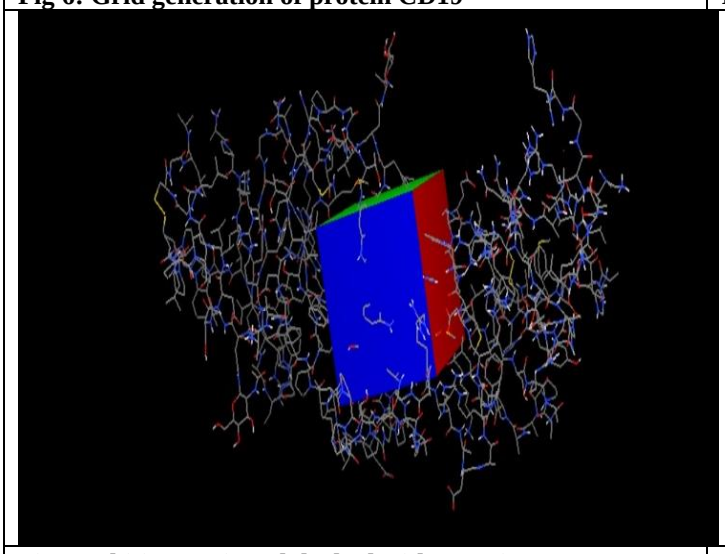


Fig 7: Grid box enclosing whole protein CD19

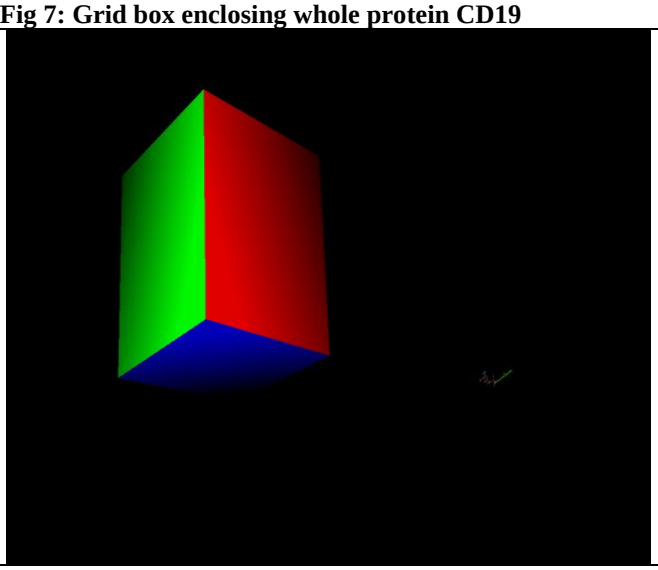


Fig 8: Chitinase Ligand docked with protein CD19

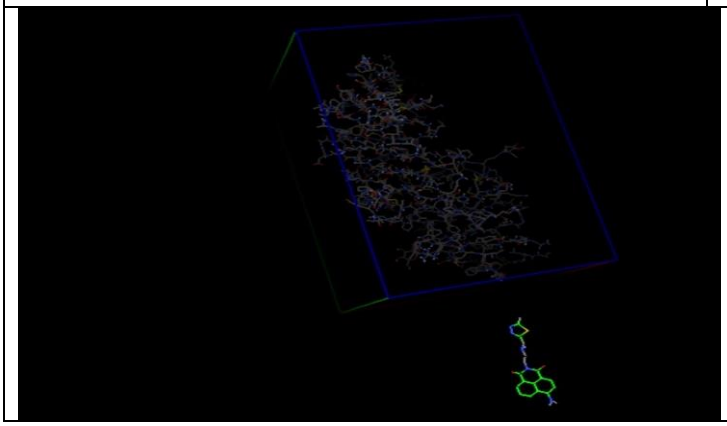


Fig 9: 3D Structure of Pyrrolnitrin ligand of Pseudomonas Fluorescens

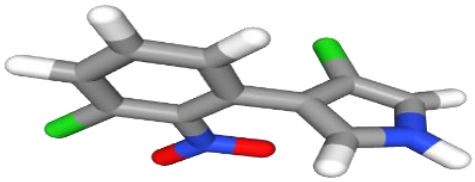


Fig 10: 3D Structure of Pyoluteorin ligand of Pseudomonas Fluorescens

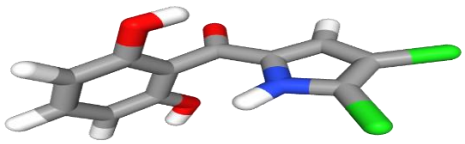


Fig11: 3D Structure of Salicylic acid ligand of Pseudomonas Fluorescens

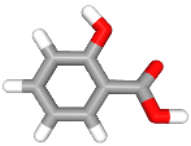
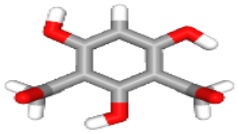


Fig12: 3D Structure of 2,4-Diacetyl phloroglucinol ligand of Pseudomonas Fluorescens



CONCLUSION

In the present analysis, secondary metabolites (chitinase, pyrrolnitrin, pyoluteorin, salicylic acid, 2,4-Diacetyl phloroglucinol) were docked successfully with target protein CD19.The binding energy of chitinase was recorded to be -6.04Kcal/mol. Therefore, it can be a potential medication for acute lymphoblastic leukaemia and it can be concluded that secondary metabolites identified from pseudomonas fluorescens may have anticancer property against leukemic blast cell lines. Moreover, the scope and applications of chitinase (secondary metabolite) are well established as anti-leukemic agent for drug targeting and consequent medical applications. Further studies can be used to design and develop novel compounds having inhibitory activity against acute lymphoblastic leukaemia.

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CONFLICT OF INTERSEST

The authors declare no conflicts of interest

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