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Review Article

ANTICANCER ACTIVITY OF SECONDARY METABOLITES OF GRAMPOSITIVE BACTERIA STREPTOMYCES COLONOSANANS (CA-256286) AGAINST COLORECTAL CANCER USING MOLECULAR DOCKING

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ABSTRACT

Colorectal cancer is the third highest pervasive rate of all cancer types worldwide and it is also one of the major causes of mortality. Chemotherapy drugs are generally limited due to various complexities, as well as the development of resistance and recurrence. Hence, scientific communities have given more significance to natural products as drugs derived from them provide better treatment compared to synthetic product. Besides, the availability of compounds derived from natural sources, especially the secondary metabolites derived from microorganism play important role in cancer treatment. In these Soil gram-positive bacteria streptomyces colonosanans (CA-256286) secondary metabolites has been known an attractive compound with the ability of anticancer activity in cancer cells. In the present study, the in-silico docking examination involved exploration of protein, 3D Structural modelling and binding energy calculation. SMAD2 (PDB id: 1 KHX) Protein was used in the molecular docking analysis of secondary metabolites such as 1,8 dihydroxy-2-ethyl-3-methyl anthraquinone, anthracycline, elaiomycin, piericidin, streptokordin for chemotherapeutic activity. The compound anthracycline had the highest binding energy scores with the target protein SMAD2 (PDB id: 1 KHX), according to molecular docking results. The findings suggest it could be used to develop new anticancer drugs. The conclusion drawn from the study should be validated by further evaluation in animals and humans

Keywords: Colorectal cancer, Soil microorganism, Secondary metabolites, Molecular docking.

INTRODUCTION

Cancer is a challenging health problem around the world with increasing urbanization and the consequent changes in life style. According to recent report by the WHO, there are now more than 10 million cases of cancer per year worldwide. The fact that about 7 million people die from various type of cancer every year, making this disease responsible for 12.5% of death worldwide, raises an overwhelming demand to develop new, more potent and effective, anticancer agent[6].

The tumour types with highest incidents were lung (12.3%), breast (10.4%) and colorectal (9.4%)[2]. Among these types of cancer, colorectal cancer is the third most common cancer and the fourth most common cause of cancer related death. In western countries, it has become a predominant cancer and accounts for approximately 10% cancer related mortality[4]. An ideal chemotherapy drug should have high specificity and ability to discriminate between cancer and normal cells but many of the anticancer drugs in use are still lacking in the drugs specificity[8]. Hence, recently researches have been conducted on complementary and alternative medicine that deals with cancer management.

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Natural products have historically and continually been investigated for promising novel drug leads for anticancer activity. Literature surveys have been shown that secondary metabolites from soil microorganisms can be introduced as anticancer agents. Therefore, over 70% of anticancer drugs are derived from secondary metabolites of soil microorganisms[14]. An impressive number of bioactive secondary metabolites have a major impact on the control of cancer which is produced by genus Streptomyces[19].

Waksman & Henrici (1943) had proposed genus Streptomyces; the genus Streptomyces is comprised of 600 species with validly published names (http://www.bacterio.cict.fr/)[1]. Streptomyces are gram positive, filamentous bacteria that are excellent saprophytes, prolific producers of extra cellular enzyme and ubiquitous in different environments, such as soil, fresh water and sea water[11,20]. The novel strain MUSC 93J was discovered and polyphasic approach demonstrated that MUSC 93J represent a novel species of Streptomyces genus, for which the name *Streptomyces colonosanans* was proposed. *Streptomyces colonosanans* was proven to exhibit as cytotoxic and antioxidant activity by various studies[10]. Hence, we preferred secondary metabolites of soil gram positive *Streptomyces colonosanans* (CA-256286) bacteria.

In view of the above-mentioned facts in the present research, molecular docking studies were conducted between secondary metabolites [Fig.1] and colorectal cancer cell lines in order to provide understandable evidence for the observed anticancer activity of the compounds. The computational bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed[18].

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: 1KHX) [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 algorithm[5].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 compound and predicting the strongest binders based on their scoring functions. Proteins, 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 SMAD2, SMAD4, TGF-β, BAX, BRAF, APC gene, WNT receptor are important markers used to identify colorectal cancer. We selected SMAD2 protein as a molecular target to prevent colorectal cancer. These bioactive compounds are secondary metabolites such as 1,8dihydroxy-2-ethyl-3-methyl anthraquinone, anthracycline, elaiomycin, piericidin, streptokordin, halichobledide D, carboxamycin, chinikomycin. Based on the availability of 3D structure from database molecular docking analysis was carried out with 5 bioactive compounds from *Streptomyces colonosanans* (CA-256286) such as 1,8dihydroxy-2-ethyl-3-methyl

anthraquinone, anthracycline, elaiomycin, piericidin, streptokordin.

These compounds were further screened against the target colorectal cancer protein SMAD2. Among the 5 compounds from *Streptomyces colonosanans* (CA-256286), the compound anthracycline showed potential binding energy of -9.56 Kcal/mol with the targeted protein SMAD2 followed by the 1,8 Dihydroxy-2-ethyl-3-methyl anthraquinone, piericidin, streptokordin, elaiomycin with the binding energies of -8.08 Kcal/mol, -6.42Kcal/mol,-5.16Kcal/mol,-4.07Kcal/mol, respectively [Table1].

Table 1: Molecular docking analysis of bioactive compounds of Streptomyces colonosanans (CA-256286) against the colorectal cancer target protein SMAD2 (PDB id: 1KHX).

S.NO	COMPOUNDS NAME	BINDING ENERGY (Kcal/mol)	RMSD(Å)VALUE
1	Anthracycline	-9.56	0.94
2	1,8 Dihydroxy-2-ethyl-3-methyl anthraquinone	-8.08	0.51
3	Piericidin	-6.42	1.00
4	Streptokordin	-5.16	0.80
5	Elaiomycin	-4.07	1.00

TABLE 2: Showing binding energy anthracycline with 1khx

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	10	−9.56	0.00	127.00	RANKING
2	1	6	−7.48	0.00	111.71	RANKING
2	2	5	−6.38	1.46	112.34	RANKING
3	1	4	−6.89	0.00	128.39	RANKING
4	1	2	−6.71	0.00	100.95	RANKING
5	1	7	−6.32	0.00	125.64	RANKING
6	1	9	−5.97	0.00	97.16	RANKING
7	1	8	−5.76	0.00	90.42	RANKING
8	1	3	−5.21	0.00	134.13	RANKING
9	1	1	−5.06	0.00	97.02	RANKING

TABLE 3: Showing binding energy1,8 Dihydroxy-2-ethyl-3-methyl anthraquinone with 1khx

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	5	−8.08	0.00	124.49	RANKING
2	1	7	−7.29	0.00	129.59	RANKING
2	2	10	−7.15	0.21	129.71	RANKING
2	3	3	−7.14	0.24	129.72	RANKING
2	4	9	−7.09	0.48	129.62	RANKING
2	5	2	−6.79	1.34	129.41	RANKING
3	1	6	−7.26	0.00	130.80	RANKING
3	2	4	−7.26	0.07	130.80	RANKING
3	3	8	−7.25	0.09	130.81	RANKING
4	1	1	−6.52	0.00	130.47	RANKING

TABLE 4: Showing binding energy Piericidin with 1khx.

Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	8	−6.42	0.00	127.91	RANKING
2	1	6	−5.67	0.00	123.92	RANKING
3	1	4	−4.70	0.00	128.44	RANKING
4	1	2	−4.02	0.00	125.14	RANKING
5	1	9	−3.99	0.00	125.66	RANKING
6	1	10	−3.99	0.00	112.35	RANKING
7	1	3	−3.96	0.00	100.24	RANKING
8	1	7	−3.57	0.00	113.22	RANKING
9	1	5	−3.51	0.00	127.09	RANKING
10	1	1	−2.96	0.00	136.31	RANKING

TABLE 5: Showing binding energy Streptokordin with 1khx

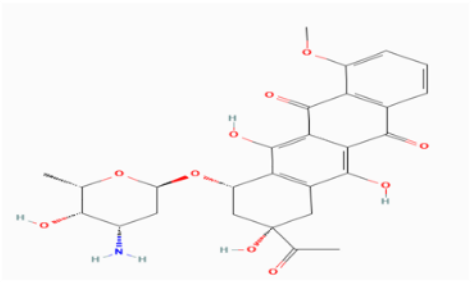
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	2	−5.16	0.00	131.32	RANKING
2	1	4	−5.10	0.00	130.93	RANKING
2	2	10	−5.09	0.05	130.92	RANKING
2	3	8	−5.07	0.34	131.12	RANKING
3	1	9	−5.01	0.00	129.44	RANKING
4	1	5	−4.75	0.00	122.99	RANKING
5	1	1	−4.64	0.00	129.64	RANKING
5	2	7	−4.64	0.18	129.56	RANKING
6	1	6	−4.59	0.00	124.92	RANKING
7	1	3	−4.53	0.00	134.94	RANKING

TABLE 6: Showing binding energy Elaiomycin with 1khx

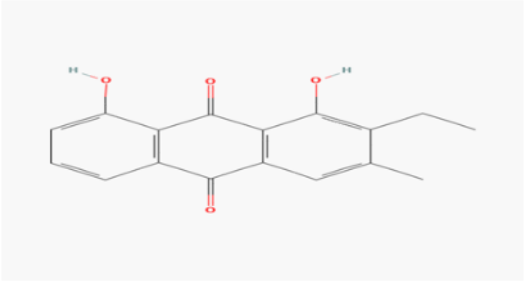
Rank	Sub-Rank	Run	Binding Energy	Cluster RMSD	Reference RMSD	Grep Pattern
1	1	9	−4.07	0.00	126.45	RANKING
2	1	2	−4.00	0.00	125.59	RANKING
3	1	6	−4.00	0.00	118.22	RANKING
4	1	8	−3.59	0.00	103.73	RANKING
5	1	10	−3.44	0.00	130.15	RANKING
6	1	5	−3.39	0.00	132.42	RANKING
7	1	4	−2.84	0.00	122.73	RANKING
8	1	3	−2.73	0.00	104.41	RANKING
9	1	1	−2.64	0.00	120.33	RANKING
10	1	7	−2.34	0.00	111.82	RANKING

Fig 1: Secondary metabolites of Streptomyces colonosanans

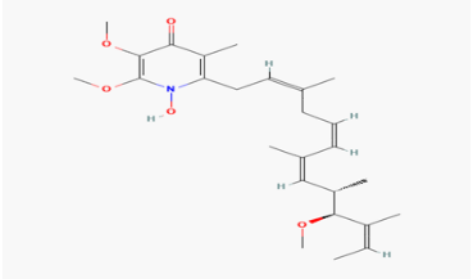
a) Anthracycline



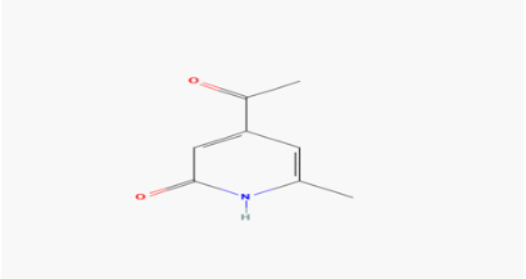
b) 1,8,Dihydroxy-2-ethyl-3-methyl anthraquinone



c) Piericidin



d) Streptokordin



e) Elaiomycin

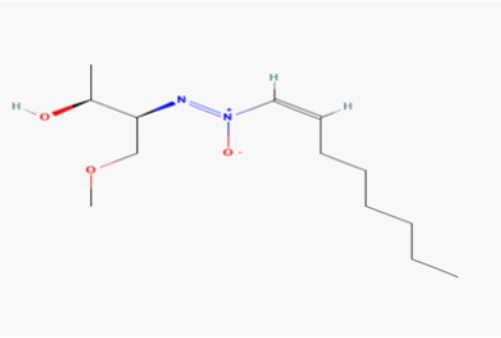


Fig 2: Protein 1KHX 3D Structure of colorectal cancer cell lines

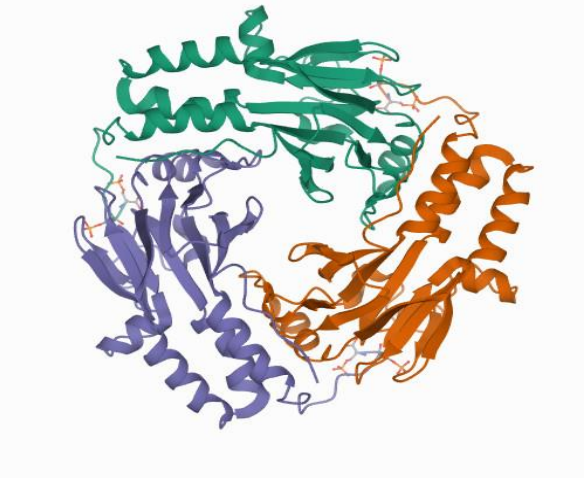


Fig 3: 1KHX Protein of colorectal cancer cell lines

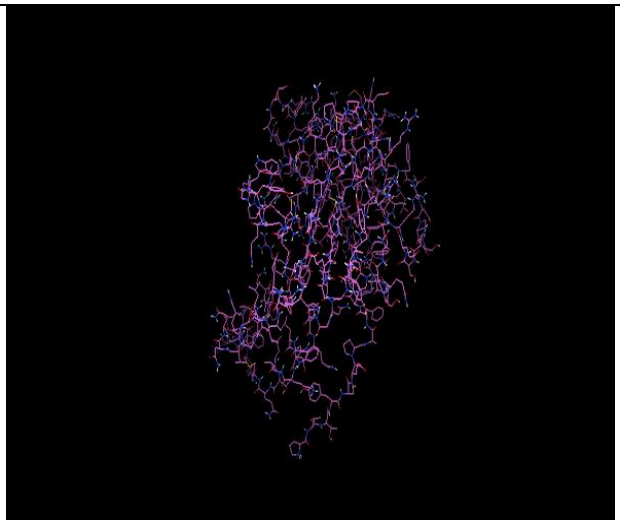


Fig 4: 3D Structure of Anthracycline Ligand of Streptomyces colonosanans

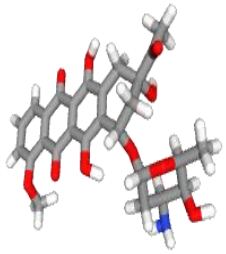


Fig 5: Anthracycline Ligand interaction with protein SMAD2

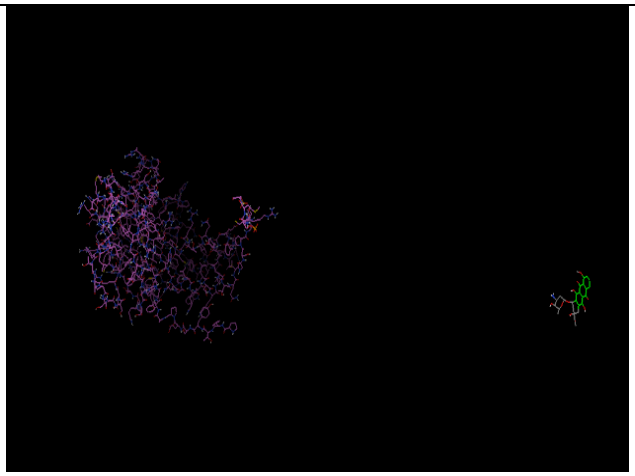


Fig 6: Grid box generation whole protein SMAD2

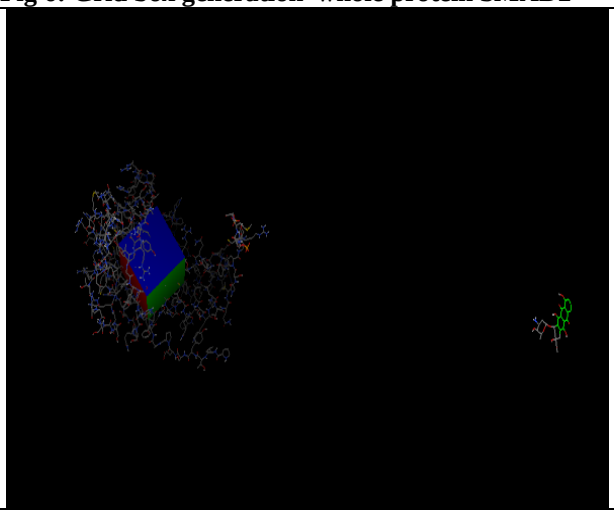


Fig 7: Grid box enclosing whole protein SMAD2

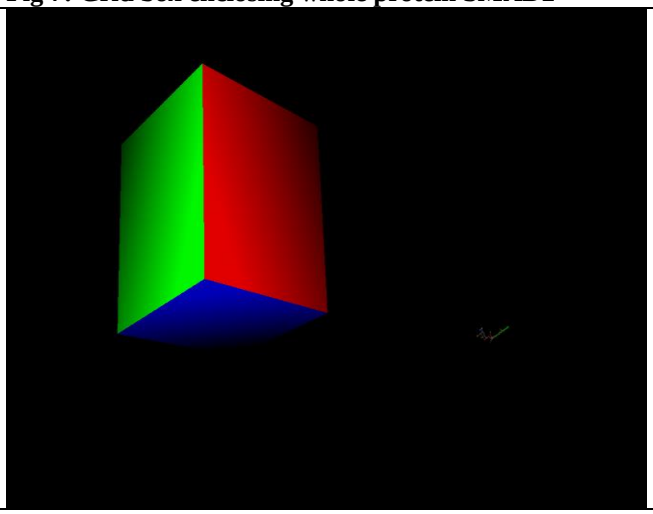


Fig 8: Anthracycline Ligand docked with protein SMAD2

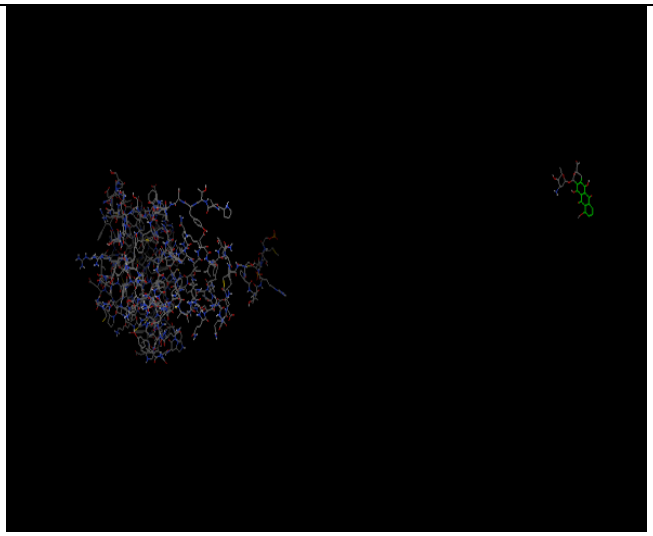


Fig 9: 3D Structure of 1,8 Dihydroxy-2-ethyl-3-methyl anthraquinone Ligand of Streptomyces colonosanans

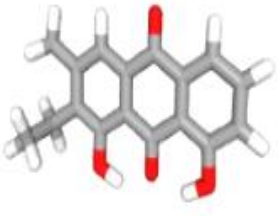


Fig 10: 3D Structure of Piericidin Ligand of Streptomyces colonosanans

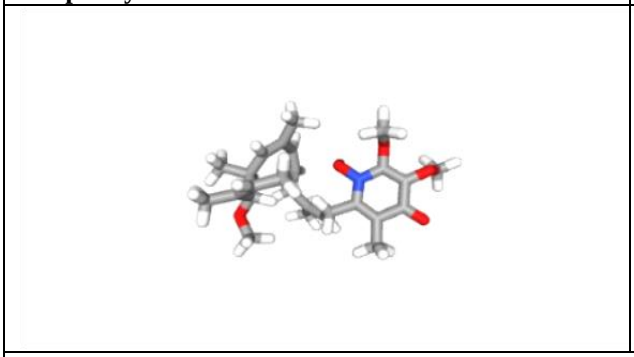


Fig 11: 3D Structure of Streptokordin Ligand of Streptomyces colonosanans

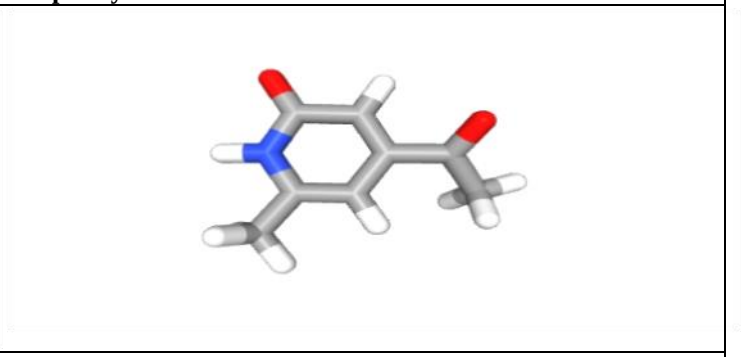
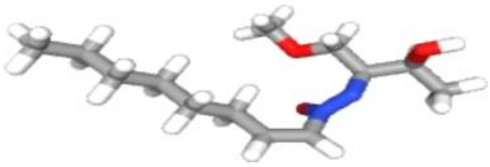


Fig 12: 3D Structure of Elaiomycin Ligand of Streptomyces colonosanans



DISCUSSION

According to Mishra et.al study (2017), it has been demonstrated that molecular docking of Everninic acid and Roccellic acid from Roccella Mantagnei exhibited free binding energy -6.65 Kcal/mol, -6.75 Kcal/mol respectively with cyclic dependent kinase-10 supstrate of Homo sapiens. Both compounds have been evaluated against human cancer cell lines where Roccellic acid shows significant activity against breast and colon cancer cell lines. Finally, concluded that cytotoxic activity of Roccella Mantagnei Thallus might be due to the presence of Roccellic acid[25]. Based on a study by Nam Q.H. Doan (2020), According to Nam Q.H. Doan (2020) Study, 40 glycyrrhetinic acid derivatives were proposed and evaluated for anti-colorectal cancer activity using molecular docking. The study demonstrates that the glycoside derivatives showed best binding affinity followed by 3β- amino derivatives, 5membered hetero cyclic ring combined derivatives had best potential[17]. Based on Asita Elengoe et.al (2020) study, allicin, epigallocatechin-3-gallate and gingeol (phytocomponents) were docked successfully with P53, APC and EGFR protein models. The study shows that P53- allicin complex had strongest binding affinity has it had lowest binding score. The phytocomponents can be used to develop more powerful structure- based drug[3].Based on Mani Suganya et.al study (2019) the proanthocyanidin compounds and correspondingly 5- FU were docked with cell cycle (CDK2 & CD4) and apoptotic (Bcl2, Bcl-XL) proteins of colon cancer. Docking results showed that PAC is having strong bonding when compared to 5- FU. Therefore, the flavonoid compounds can be considered as an effective anticancerous promoters against human colon cancer cells[13].

In this study, Autodock software was used to dock protein SMAD2 with microbial soil secondary metabolites of streptomyces colonosanans. The binding scores of secondary metabolites docked with colon cancer cell line protein were -9.56 Kcal/mol, -8.08Kcal/mol, -6.42Kcal/mol, -5.16Kcal/mol, -4.07Kcal/mol. The more negative docking score indicates stronger binding between protein and Ligand. According to the lowest binding, the anthracycline had strongest binding affinity with protein SMAD 2. Hence, the secondary metabolites *streptomyces colonosanans*

(1,8dihydroxy-2-ethyl-3-methyl anthraquinone, Anthracycline, Elaiomycin, Piericidin, Streptokordin) can considered to treat colorectal cancer but further evaluation in laboratory animals is needed.

CONCLUSION

In the present analysis, secondary metabolites (1,8dihydroxy-2-ethyl-3-methyl anthraquinone, anthracycline, elaiomycin, piericidin, streptokordin) were docked successfully with target protein SMAD2. The anthracycline was recorded to be -9.56 Kcal/mol. Therefore, it can be a potential medication for colorectal cancer and it can be concluded that secondary metabolites identified from *streptomyces colonosanans* may have anticancer property against colorectal cancer cell lines. Further studies can be used to design and develop novel compounds having inhibitory activity against colorectal cancer.

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