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RESEARCH ARTICLE

DOCKING STUDIES FOR EXPLORING THE ANTI-CANCER POTENTIAL OF BIOACTIVE COMPOUNDS

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Abstract

Cancer continues to be a leading cause of global morbidity and mortality, necessitating innovative approaches for drug discovery and development. In this study, we employed molecular docking simulations to investigate the potential anti-cancer properties of bioactive compounds against critical oncogenic targets. A diverse set of bioactive compounds, sourced from natural products and synthetic libraries, were selected for their known biological activities and structural diversity.While cancer diagnoses have been documented for a century, the root causes remained elusive to physicians. This study aimed to pinpoint potential antitumor compounds within Stevia rebaudiana. Through GC-MS analysis, fifteen compounds were detected in the leaves. In silico analysis of these bioactive compounds against PRAD1 was conducted to assess their anti-cancer potential. Docking outcomes highlighted Tetradecanoic acid and Stigmastan-3,5-diene as the most promising candidates bound to PRAD1.The identified lead candidates offer promising avenues for the development of novel anti-cancer therapeutics, emphasizing the importance of integrating computational approaches in early-stage of drug discovery.

Introduction:-

Cancer has unfortunately become a commonplace ailment, necessitating extensive research and patience for effective treatment[1]. Decades of epidemiological investigations have underscored the exceptional danger posed by tobacco use[2]. The development of cancer is known to trigger various chronic conditions, often in connection with ROS scavengers and antioxidant enzymes[3]. Pneumonia, influenza, and enduring respiratory symptoms such as coughing and wheezing, which, while not lethal on their own, can significantly diminish quality of life[4].Accumulating evidence suggests that an imbalance between caloric intake and physical activity may be linked to an elevated risk of breast cancer[5]. Additionally, obesity is associated not only with hormone-related cancers but also with the development of other forms such as renal cell, esophageal, and colon cancer[6].In addition to increasing the risk of developing hormone-related cancers[7],obesity[8] is also associated with the development of other types of cancer[9], such as renal cell[10], esophageal[11], and colon cancer[12].Doll identified a diet high in fatsas a possible contributing cause to the development of breast cancer[13]. Probiotics are also associated with various therapeutic properties such as improved immune function and fewer adenomas and colon cancers[14].PRAD1 (previously D11S287), appears to contribute to parathyroid tumorigenesis in a fashion

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analogous to activation of C-MYC or BCL-2 by rearrangement with tissue-specific enhancers of the immunoglobulin genes in B-lymphoid neoplasia[15]. In this work, we have focused our discussion on Stevia rebaudianaanticancer efficacy and associated molecular mechanisms.

Methods:-

Preparation of Stevia rebaudianasmoothie

Stevia rebaudianaleaves wereprocured froma local market located at Kanpur, Uttar Pradesh. Leaves were meticulously cleaned using distilled water to eliminate any dust particles. Subsequently, they were processed into a smoothie[16] using a mortar and pestle, and the resulting mixture was carefully transferred into a sterile test tube. Following this, the tubes underwent centrifugation at 8,000 rpm for a period of 3 to 5 minutes. This process led to the separation of the supernatant, which was then appropriately stored for subsequent GC-MS analysis.

Analysis of bioactive compounds

Identification of compounds in the extract was conducted using an Agilent GC-MS-5975C instrument operating in electron energy mode at 70 eV. A capillary column (CB-MS) with an inner diameter of 0.32mm, a length of 30m, and a coated material film thickness of 0.25 µm was employed. The GC analysis was carried out in splitless mode within a temperature range of 220 to 270°C. The carrier gas, helium, was maintained at a constant flow rate of 1 ml/min. Mass spectra were obtained through a comparison of retention times and peak areas with those of authentic compounds[17].

PRAD1Active site Identification

The structure of PRAD1(PDB: 1GJH) was retrieved from the PDB database and unnecessary chains, heteroatoms were removed using SPDBV software, hydrogens were added to the protein and used for active site identification. The active site of PRAD1 of Homo sapiens was identified using the CASTp server. A new program of CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings[18].

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on a genetic algorithm whichallows partial flexibility of protein and full flexibility of ligand. The compounds identified in GC-MS are docked to the active site of the PRAD1 of Homo sapiens. The interaction of the compounds with the active site residues is thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of the islands (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A° (dH-X) for hydrogen bonds and 6.0A° for Vander Waals were employed. During docking, the default algorithm speed was selected and the ligand binding site in the targets wasdefined within a 10A° radius with the centroid as CE atom of active residues. The number of poses for each inhibitor was set to 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A° RMSD. After docking, the individual binding poses of compounds were observed and their interactions with the protein were studied. The best and most energetically favourable conformation of ligands was selected[19].

Results And Discussion:-

GC-MS analysis

Fifteen components in ethanol extract of Stevia rebaudianaleaf were identified.

Name of the compound	Retention Time	Peak Area %
Propane, 1,1-diethoxy-	4.77	9.84
Cyclononanone	20.08	14.0
Tetradecanoic acid	12.74	22.8
β-Sitosterol acetate	24.72	4.56
γ-Sitosterol	31.30	4.14
Cholesta-4,6-dien-3-ol, (3β)-	23.64	3.84
t-Butyl hydrogen phthalate	25.19	2.22
Eicosanoic acid, phenylmethyl ester	22.33	4.02

Benzamide, N-[2-(5-methoxy-2-methyl-1H-indol-3- yl)ethyl]-3-methyl-4-nitro	32.81	3.67
3,4-Dihydroxy-α-(isopropylaminomethyl)-benzyl alcohol (isoproterenol)	10.35	2.11
3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	8.26	9.76
Cyclohexasiloxane, dodecamethyl-	6.64	4.44
9,19-Cyclolanostan-3-ol, acetate, (3β)-	34.16	4.70
Stigmastan-3,5-diene	32.33	13.96
Benzeneacetic acid, α,3,4-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester	5.84	4.72

From the PDB databank, the PDB files were collected and the final stable structure of the PRAD1 of Homo sapiens obtained is shown in Figure 1. The ligands present in the crystal structure were removed along with hetero atoms for docking studies.

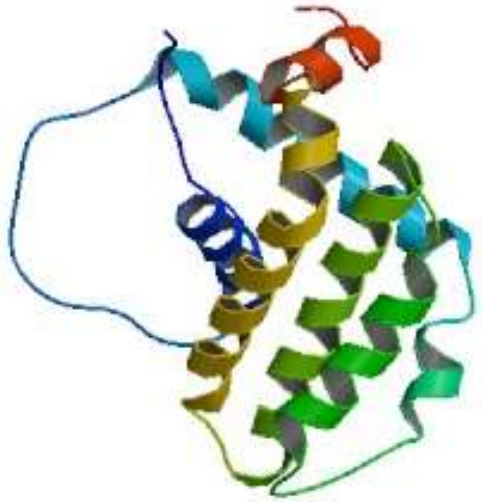

Figure 1:- Structure of PRAD1 retrieved from protein data bank with seven helices.

Active site Identification

After the final model was built, the possible binding sites of PRAD1 were searched based on the structural comparison of the template and the model build with CASTP server as shown in Figure 2. In fact, from the final refined model of PRAD1 domain using SPDBV program, it was found that secondary structures are highly conserved and the residues shown below

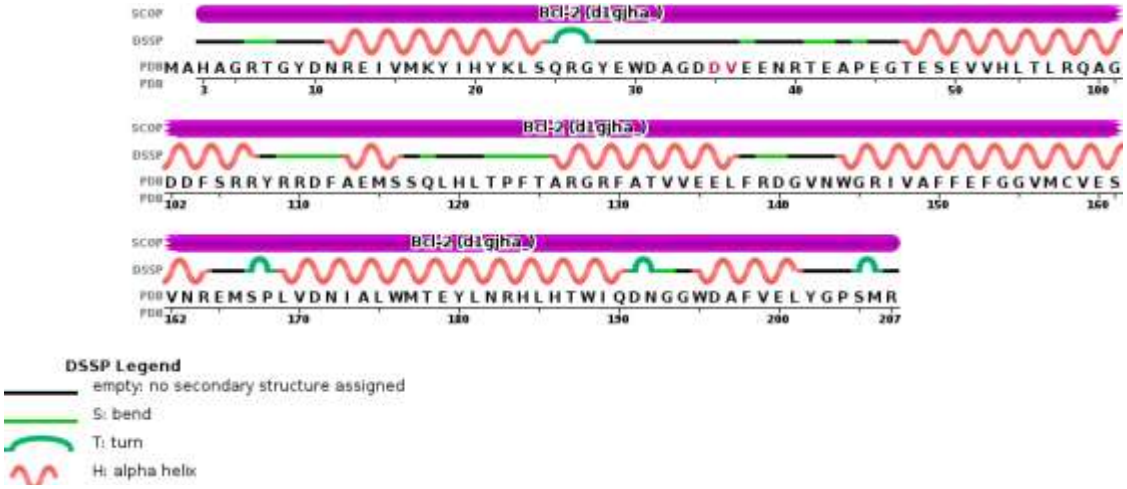

Figure 2:- Amino acids in the active site region (red colour) of the PRAD1protein.

Docking of inhibitors with the active site

Docking of the compounds with PRAD1was performed using GOLD 3.0.1, which is based on a genetic algorithm. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystallized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking were converted in 3D with SILVER. To this set, the substrate corresponding to the protein was added. Docking of the best inhibitor with the active site of protein showed the activity of the molecule on protein function.

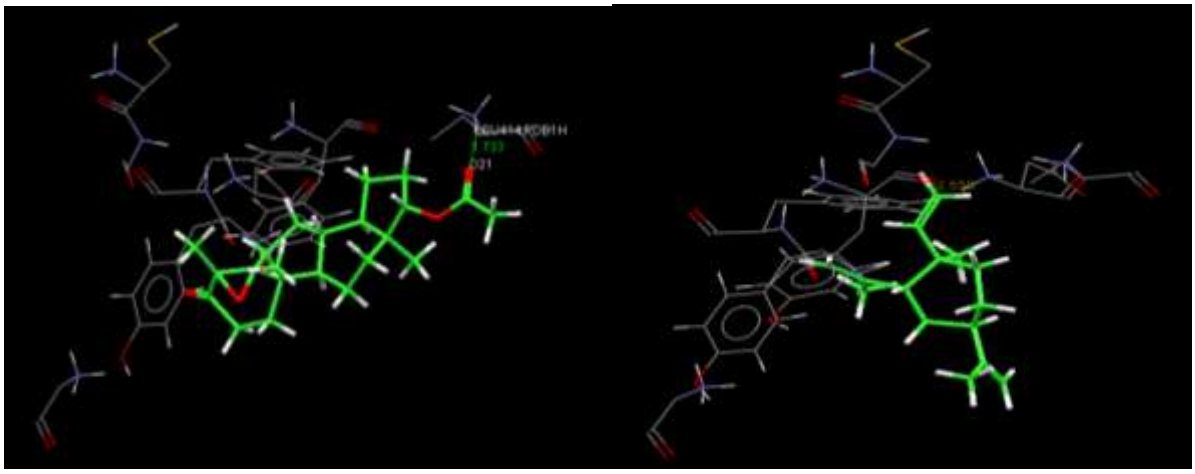

Figure 3:- Tetradecanoic acid and Stigmastan-3,5-diene docked to PRAD1 active site.

Tetradecanoic acid showed docking energy of 26.52K.cal/mol and stigmastan-3,5-diene of 28.24K.cal/mol with PRAD1. Tetradecanoic acid docked to LEU414 with a bond length of 1.733A^{o} and stigmastan-3,5-diene docked to LEU414 with a bond length of 2.559A^{o} respectively.

Conclusion:-

From the studies, we conclude that GC-MS analysis identified twentyphytocompounds from Stevia rebaudianaextract. The identified phytocompounds were checked for their anti-cancer activity using insilico method.PRAD1 protein was retrieved from the database and its active site was identified using the CASTp server. Allphytocompounds were docked to the PRAD1 for their anti-cancer activity, out of those twenty, Tetradecanoic acid showed docking energy of 26.52K.cal/mol and stigmastan-3,5-diene of 28.24K.cal/mol with PRAD1.From these docking studies weconclude that among the phytocompounds identified, Tetradecanoic acid and stigmastan-3,5-diene have good PRAD1 inhibitory activity.

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