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Molecular docking studies of bioactive compounds from *Stevia rebaudiana* for its anti-cancer activity

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Abstract

Background: Although cancers have been diagnosed for the past 100 years, physicians were at a loss to explain the underlying causes of the disease. Aim: The purpose of this study was to identify compounds in *Stevia rebaudiana* that could have antitumor activities. Methods: GC-MS analysis revealed the presence of fifteen compounds in the leaf. Results: Insilico analysis of bioactive compounds with PRAD1 is done to check anti-cancer activity. Conclusion: Docking results showed Tetradecanoic acid and Stigmastan-3,5-diene as best docked to the PRAD1.

Keywords: Anticancer, *Stevia rebaudiana*, Tetradecanoic acid, Stigmastan-3,5-diene, Docking studies, Alpha-Glucosidase

INTRODUCTION

Cancer has become ordinary in the population while the treatment is onerous, demanding a lot of patience and research[1]. Decades of epidemiologic research have demonstrated that tobacco is a uniquely hazardous substance[2]. Development of cancer is known to cause various chronic diseases in association with ROS scavengers and antioxidant enzymes, such as pneumonia, influenza[3], and various persistent respiratory symptoms[4] such as cough and wheezing which, while not deadly by themselves, may greatly reduce quality of life[5]. A growing body of evidence suggests that excess caloric intake in relation to physical activity may be associated with breast cancer risk[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]. Armstrong and Doll[13] identified a diet high in fats as a possible contributing cause to the development of breast cancer. However, studies also suggest that the consumption of fat alone is not a contributing cause, and identify rather the total number of calories consumed, especially those consumed in early life. Probiotics[14] are also associated with various therapeutic properties such as improved immune function and fewer adenomas and colon cancers. PRAD1 (previously D11S287), appears to contribute to parathyroid tumorigenesis in a fashion 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 rebaudiana* anticancer efficacy and associated molecular mechanisms.

METHODS

Preparation of *Stevia rebaudiana* smoothie

Stevia rebaudiana leaves were were procured from Paraman Food works, Amazon. Leaves were cleaned thoroughly with distilled water to remove dust and were made into a smoothie[16] with the help of a mortar and pestle and collected into a sterile test tube. These tubes were centrifuged at 8,000 rpm for 3-5 minutes and the supernatant was separated and stored for GC-MS analysis.

Analysis of bioactive compounds

GC-MS-5975C (Agilent) operating in electron energy mode at 70 eV is used for identification of compounds in the extract. Capillary column CB-MS of inner diameter (30m·0.32mm), of 0.25 lm film thickness of coated material was used. GC was performed in the splitless mode from 220 to 270^{0}C. The flow rate of carrier gas (helium) was maintained at 1ml/min. Mass spectra were taken by comparing the retention times and peak area with those of authentic compounds[17].

PRAD1 Active 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[19] which is based on a genetic algorithm which allows as 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 are 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 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.

RESULTS AND DISCUSSION

GC-MS analysis

Fifteen components in ethanol extract of *Stevia rebaudiana* leaf 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.

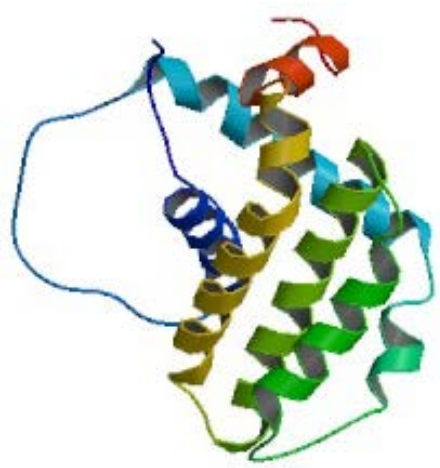

Figure 1: Structure of PRAD1 retrieved from protein data bank with seven helices

Active site Identification

After the final model was built, the possible binding sites of PRAD1 was 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 in figure 2

Docking of inhibitors with the active site

Docking of the compounds with PRAD1 was 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. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was 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.

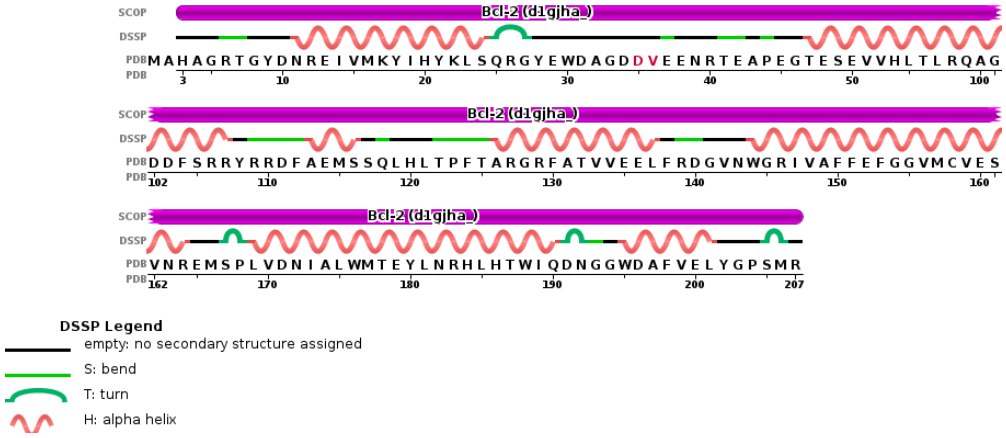

Figure 2: Amino acids in the active site region (red colour) of the PRAD1protein

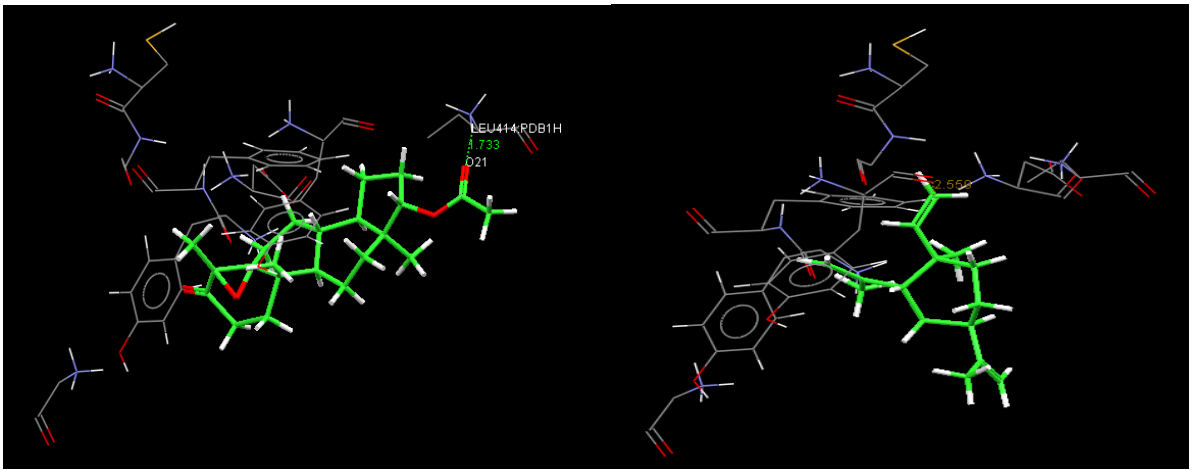

Figure 3: Tetradecanoic acid and Stigmastan-3,5-diene docked to PRAD1 active site

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 twenty phytocompounds from *Stevia rebaudiana* extract. 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. All phytocompounds 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 we conclude that among the phytocompounds identified, Tetradecanoic acid and stigmastan-3,5-diene have good PRAD1 inhibitory activity.

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CONFLICT OF INTEREST

Nil

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