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GC–MS ANALYSIS, *IN SILICO ANALYSIS* OF PHYTOCOMPOUNDS FROM *CROTON BONPLANDIANUM* AGAINST LUNG CANCER

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ABSTRACT

This study aimed to identify putative anti-cancer phytocompounds using gas chromatography-mass spectrometry in an ethanolic extract of the whole Croton bonplandianum plant. These molecular docking investigations aimed to determine the impact of the discovered phytocompounds on a protein specific to lung cancer. The ADMET capabilities of the discovered phytocompounds were also assessed by testing their pharmacokinetic characteristics. The docking software PyRx was used for molecular docking studies, and SwissADME was used to investigate the pharmacokinetic features of phytocompounds. Based on their pharmacokinetics and fallow Lipinski's rule of properties, for molecular docking, seven phytomolecules were selected. A pair of these chemicals demonstrated exceptional binding affinity for a protein that is specific to lung cancer. Their molecular weights are 7.2 kcal/mol for Alpha-tocospiro A and 1, 4-Dibutyl benzene-1,4- dicarboxylate, respectively. These findings also support the use of molecular dynamics modeling to confirm the interaction between phytocompounds and proteins. This study's findings suggest that phytocompounds found in an ethanolic extract of the Croton bonplandianum entire plant may one day be useful in the treatment of lung cancer.

KEYWORDS: *Croton bonplandianum*, molecular docking, molecular dynamics, lung cancer.

INTRODUCTION

Herbal medicines have been a conventional as well as traditional part of drug discovery, formulation, and development. All plants produce chemical constituents, as part of their normal metabolic activities. Plants serve as a source for both natural as well as synthetic drugs. Croton bonplandianum is a Monoecious woody shrub, which is 1-5m high, but more usually 30-40cm, with whorled branches. According to reports, this plant is unique to Paraguay, southern Bolivia, southwestern Brazil, northern Argentina, Pakistan, India, Bangladesh, and South America. It is frequently employed in India, which is in West Bengal's Sub-Himalayan area. It is fully spread in West Bengal's Sub-Himalayan region in India.[1,3] This plant is called Ban Tulsi because its leaves and flower cymes resemble those of Tulsi. Locally in the west Sub-Himalayan region of Bengal.[4-5] A key part of drug development for a futuristic method will continue to be the naturally occurring molecules from herbal sources with medicinal potential, which have led to numerous chemotherapeutics. The use of phytocompounds that are derived from plants that have chemopreventive properties in the treatment of cancer is becoming more and more supported. Analgesic, immunosecretory, antioxidant, antiviral, anti-inflammatory, and anti-ulcerogenic properties were found in pharmacological investigations of the whole plant of C. bonplandianum.[6-9]

Modern drug design makes use of molecular docking methods to comprehend the interaction between a medication and its receptor. Because computational tools provide insight into the process of drug-receptor interaction, they significantly promote and encourage the discovery of new, more effective inhibitors.[10-11] The public can anticipate a substance's physicochemical characteristics, (ADME) and pharmacokinetic features using a web tool called the Swiss ADME web tool. These constitute significant initiatives for carrying out clinical testing.[12-15] Amino acid's dominant binding model with a protein having a known three-dimensional structure is predicted through ligand-protein docking. Finding putative phytocompounds (ligands) from the entire plant water-soluble extract of C. bonplandianum was the primary goal of this work.[16,17] The ligands were shown to be related to the Tumor Protein P53 (TP53) gene, KRAS, and Caspase 9 lung cancer susceptibility proteins. As a potential treatment for breast cancer, this might be investigated further as a future avenue to

developing a promising novel medicine and cutting down on the time required for drug discovery.[18,20]

MATERIALS AND METHODS

Plant collection and authentication- Collection and authentication of plant material Fresh and disease-free leaves C. bonplandianus plant were collected from the medicinal garden of csjmu, Kanpur Uttar Pradesh, India. The plant specimen was recognized and verified by Dr. Muhammad Arif (Associate professor), Dept. Pharmacognosy & Phytochemistry Faculty of Pharmacy, Integral University, Lucknow, and U.P, INDIA 226026. The accession no. for the specimen is IU/PHAR/HRB/24/15

Preparation of aqua-ethanolic extract

About 200 g of the dried powder of samples was weighed, soaked, and dissolved in 600 ml of 95% ethanol in a 1000-ml conical flask and placed for cold maceration for about 1 day at normal room temperature. The flask was tightly plugged with absorbent cotton and aluminum foil and was stirred periodically using a rotary shaker for 24 hours. The extract was filtered using Whatman no. 1 filter paper. The final yield (10 g) of filtered extracts in the form of concentrated paste was used for further study.

Identification of phytochemical constituents Using Gas Chromatography

The C. bonplandianum extract executed a qualitative GC-MS study to find the phytochemical components. The Shimadzu Model GC MS-TQ8040 coupled with MS was used to analyze the ethanolic extract. A capillary column (30 m × 0.25 mm ID, 0.25 u df) and SH-Rxi-5Sil MS (5% biphenyl, 95% dimethyl polysiloxane) were installed in the device. The helium flow rate was 1.61 mL/minute, and the temperature ranged from 60°C (1′) to 260°C for 15 minutes, with a rate of 5°C/min. There was a 0.7 kV detector voltage and a 220°C ion source temperature control. The ethanol was added to the extracts before they were put into the column. Analyzing mass spectra of unknown substances and comparing them to those in the Wiley GC/MS Library, Mass Finder Library, and Adams Library helped to identify them.

In Silico Studies

Ligand Selection

The ethanolic solution of C. bonplandianum was subjected to GC–MS analysis, which revealed 36 components. An analysis was done on each component. Utilizing data from the PubChem database, the 3D structures of every chemical were obtained for this investigation.

Selection of Target Protein

Lung cancer target protein The literature contained information on KRAS, caspase-9, and tumor protein T53. This target protein's three-dimensional structure was obtained from the protein database. Tumor protein P53- (PDB ID: IA1U), caspase 9 (PDB ID: 1JXQ), and KRAS (PDB ID: 4EPW) are the PDB IDs for these three proteins.

Molecular Docking Studies

We utilized the PyRx software, which may be found at http://pyrx.sourceforge.net/downloads, to perform the molecular docking procedure. The three-dimensional structures of the receptors were imported into the PyRx application before docking using the pdb file format. The phytomolecule ligands were after that inputted into the computer in a standardized data format (.sdf) and subjected to a reduction technique aided by the program's Open Babel component. After that, we transformed the receptor and ligand files into .pdbqt format using Autodock4. It is often assumed that while docking, receptors remain rigid and ligands exhibit flexibility. The grid box parameters default to X, Y, and Z coordinates. The docking calculations were executed using Autodock Vina. After the docking method was completed, the complexes with the lowest binding energies (in kcal/mol) were chosen for additional study. We utilized Discovery Studio 2022 to see how the phytomolecules interacted with the receptor.

Molecular dynamics simulation

For the purpose of molecular dynamics modeling, the *C. bonplandianum* phytoconstituents piperlonguminine was studied using the programs Tumor protein P53- (PDB ID: IA1U), Caspase 9 (PDB ID: 1JXQ) and KRAS (PDB ID: 4EPW). Many carcinogenic target proteins were strongly bound to by these phytoconstituents. Our MD simulations were executed using the SwissParam and Molecular Dynamic Simulation software packages. After that, you may utilize these to code, coordinate files, and save them in the. reformat, which the CHARMM force field can identify. Protein coordinate and topology files compatible with the CHARMM force field were also generated using the pdb2gmx function of GROMACS 2023. After that, the water molecules of TIP3P were placed in a triclinic box. In this investigation, we examined every possible combination of protein and ligand using Periodic boundary conditions. The spacing of 1 nm was maintained consistently between the solute and the box wall. Choosing an energy minimization strategy with a maximum step size of 50,000 allowed for further system relaxation. Using an equilibrium threshold of 100.0 kJ mol−1 nm−1, the

steepest descent technique was used to carry out the energy reduction steps. After two equilibration cycles of 1000 ps, the system was cooled to 310 K and 1 bar to replicate physiological conditions. There were two equilibrations performed: one in the NVT ensemble (where volume, temperature, and number of particles were kept constant) and the other in the NPT ensemble (where pressure, temperature, and number of particles were kept constant). In particular, the Parrinello-Rahman pressure coupling technique and the velocity-rescale thermostat were used to maintain a steady pressure and temperature. Finally, 1 bar of pressure, 310 K, and 100 ns of integration time were used to execute the manufacturing stage of the MD simulation. Complex coordinates and energy data were saved every 10ps to analyze measures such as total energy, the root mean square deviation (RMSD), and the mean amount of bonds of hydrogen formed during the simulation. The temperature and pressure were maintained constant by utilizing the velocity-rescale thermostat when combined with the Parrinello-Rahman pressure coupling technique. Chemical property graphs showing H-bonds, root mean square deviation fluctuation (RMSF), radius of gyration (Rg), and solvent accessible surface area (SASA) were used to analyze the MD simulation results, building on prior work in the field. Xmgrace 5.1.25 was used to generate the graphs and figures.

RESULT

GC-MS Analysis

Croton bonplandianum ethanolic extract's phytochemical spectrum profile as determined by GC-MS data was contrasted with known substances kept in the GC-MS-attached NIST library. It was possible to identify 36 different phytochemical structures. Table 1 displays the phytomolecules' retention duration, compound name, percentage of area, and base m/z. In Figure 1, the C. bonplandianum GC–MS chromatogram is displayed.

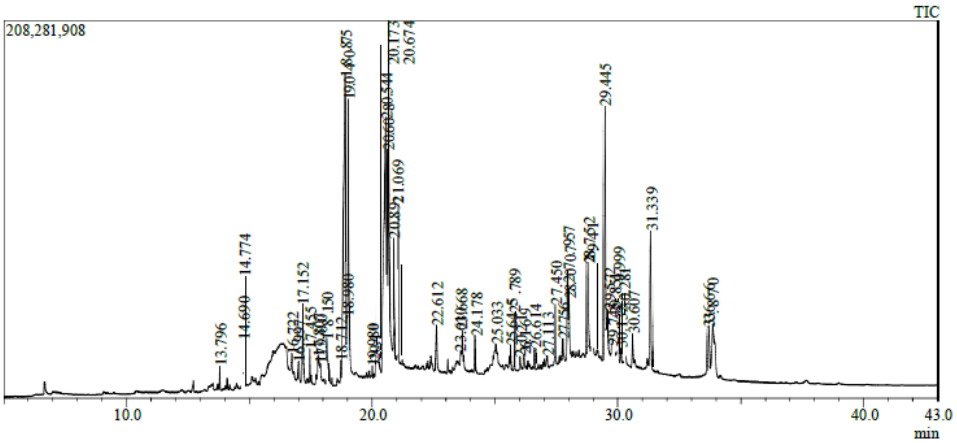


Figure 1: GC-MS profiling of ethanolic extract of *C. bonplandianus.*

These, the target protein has interacted with seven different chemicals. Three of these substances exhibited exceptionally high binding affinities with the target protein.

Table 1: Phytochemical characterization of *Croton banplandadium*.

S. No	R. Time	Area%	Chemical Name	Base m/z
1	14.690	0.87	Diethyl Phthalate	149.05
2	14.774	2.35	1,4-Benzenedicarboxylic acid, diethyl ester	149.95
3	16.722	0.45	Tetradecanoic acid	43.05
4	17.455	0.47	Neophytadiene	95.05
5	17.807	0.60	Caffeine	194.10
6	18.150	1.70	Lidocaine	86.15
7	18.712	0.31	1,4-Dibutyl benzene-1,4-dicarboxylate	149.00
8	18.875	11.87	l-(+)-Ascorbic acid 2,6-dihexadecanoate	60.00
9	18.980	1.15	Hexadecanoic acid, ethyl ester	88.00
10	19.040	5.12	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl	88.95
11	19.980	0.22	Heptadecanoic acid, ethyl ester	88.00
12	20.100	0.22	Phytol	71.05
13	20.173	7.49	Oxirane, hexadecyl-	123.15
14	20.554	11.05	Trichloroacetic acid, tridec-2-ynyl ester	93.05
15	20.608	3.72	Linoleic acid ethyl ester	81.05
16	20.674	8.45	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,	93.05
17	20.892	2.43	Octadecanoic acid, ethyl ester	88.05
18	22.612	1.07	12-Oxododecanoic acid, ethyl ester	88.00
19	24.178	0.55	Docosanoic acid, ethyl ester	88.05
20	25.645	0.37	Ethyl tetracosanoate	88.05
21	25.789	0.80	Squalene	69.05
22	26.021	0.29	Alpha.-Tocospiro A	419.35
23	27.113	0.34	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10	69.05
24	27.756	0.46	1-Hexacosanol	97.10
25	27.957	1.53	beta.-Tocopherol, O-methyl-	430.35
26	28.007	1.15	Cholesterol	81.05
27	28.752	2.15	Campesterol	315.30
28	28.941	2.18	Stigmasterol	83.05
29	29.445	6.35	gamma.-Sitosterol	329.30
30	29.572	1.69	beta.-Amyrone	218.20
31	29.745	0.93	Lanost-8-en-3-ol, (3.beta.)-	395.35
32	29.851	1.12	beta -Amyrin	218.20
33	29.999	1.70	24-Norursa-3,12-diene	218.20
34	30.132	0.31	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	95.05
35	30.607	0.59	gamma.-Sitostenone	124.10
36	31.339	3.31	Phytyl tetradecanoate	123.10

1. Molecular docking interaction of selected phytomolecules with Tumor protein P53- (PDB ID: IA1U)

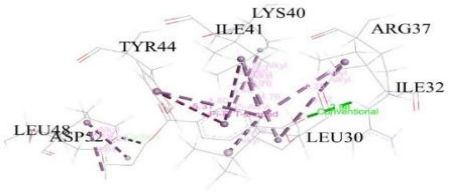
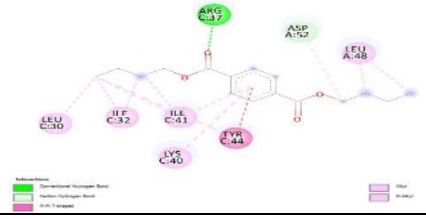
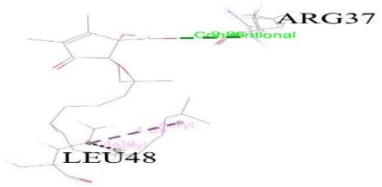
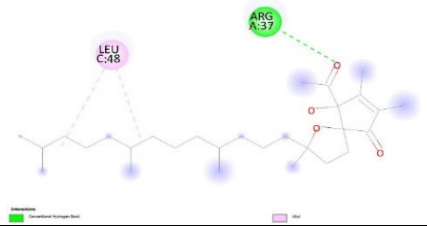
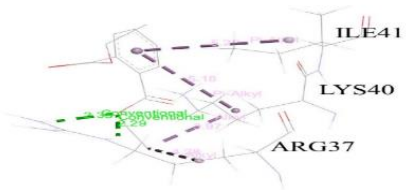
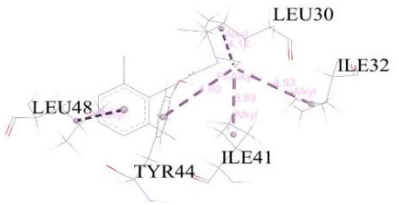
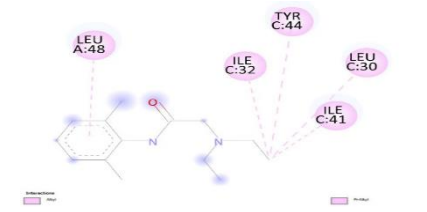
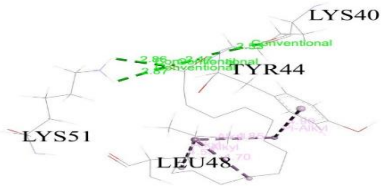
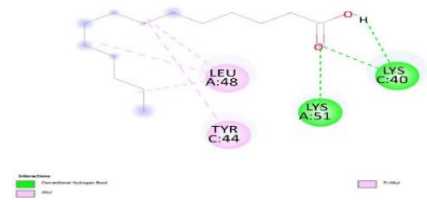
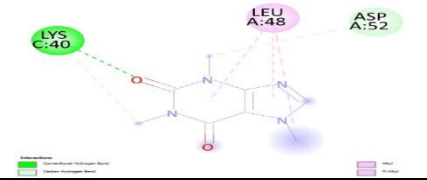
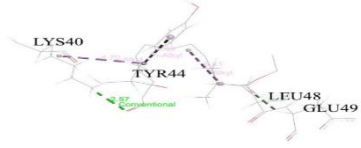
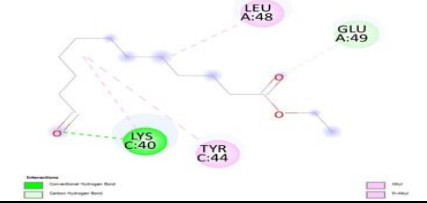
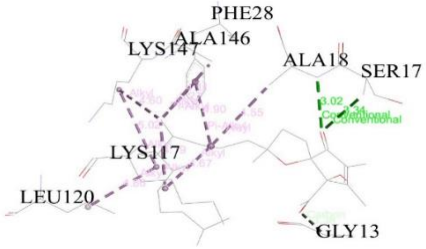
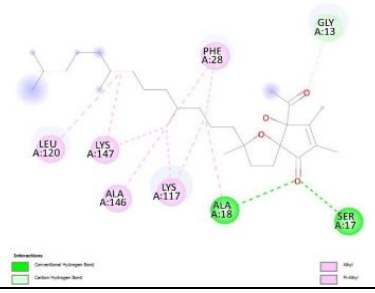
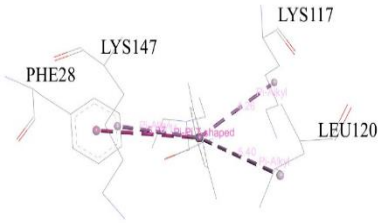
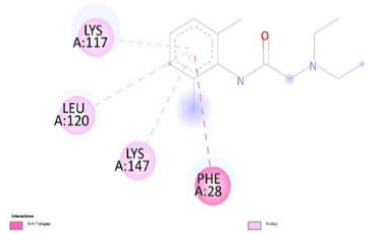
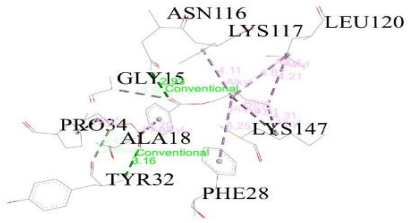
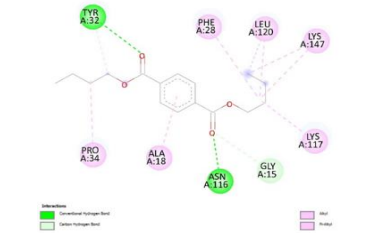
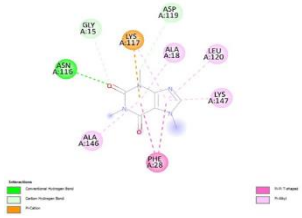
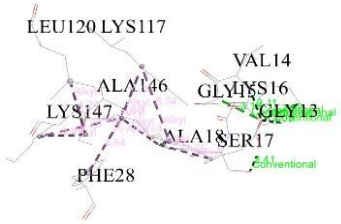
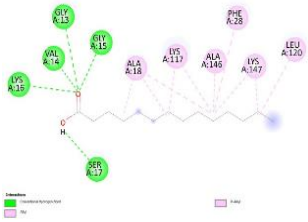
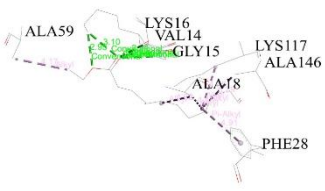
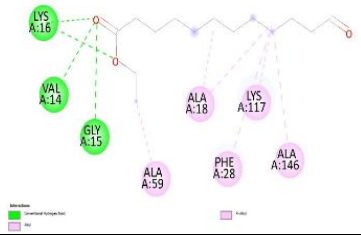
S.NO	KRAS - PDB ID - 4EPW	PyRx Binding Affinity	3D Interaction	2D Interaction
1.	16066	-5.5		
2.	21674156	-5.4		
3.	6781	-4.7		
4.	3676	-4.6		
5.	11005	-4.3		
6.	2519	-4.3		
7.	560304	-3.8		

Table 5: molecular docking interaction of selected phytomolecules with KRAS (PDB ID: 4EPW).

S.NO	COMPOUND CID	PyRx Binding Affinity	3D Interaction	2D Interaction
1.	21674156	-6.9		
2.	3676	-6.1		
3.	16066	-6		
4.	2519	-6		
5.	11005	-5.9		
6.	560304	-5.8		

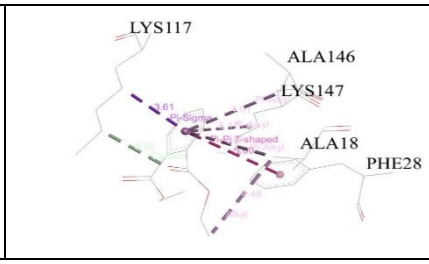
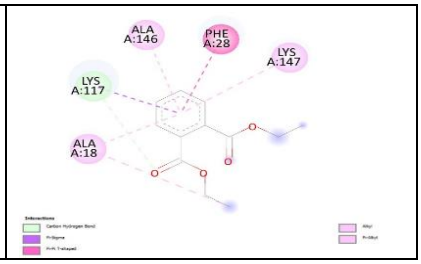
7.	6781	-5.6		

Table 6: Molecular docking interaction of selected phytomolecules with Caspase 9 (PDB ID: 1JXQ).

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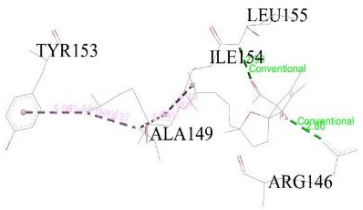
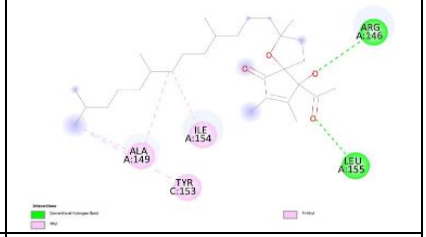
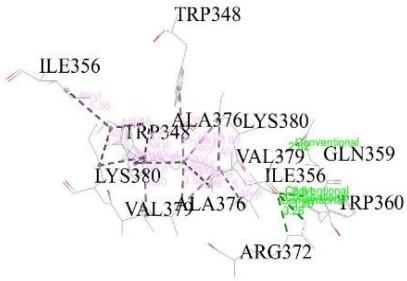
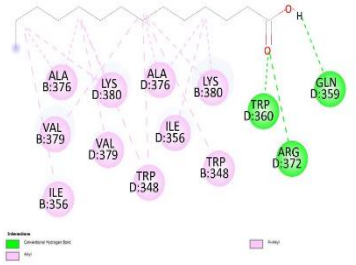
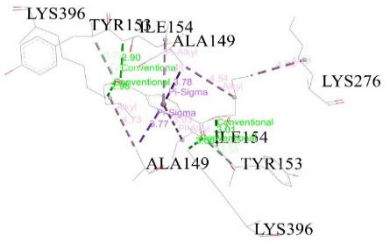
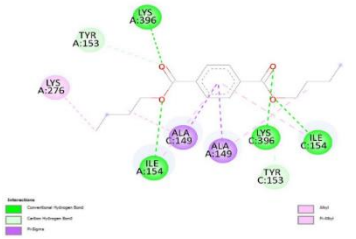
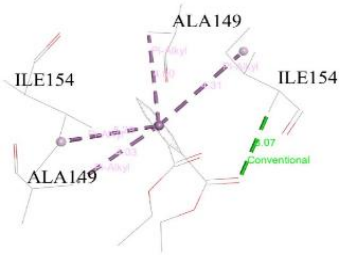
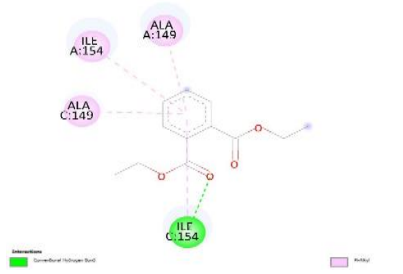
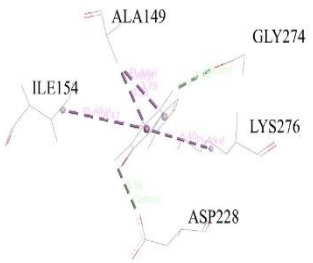
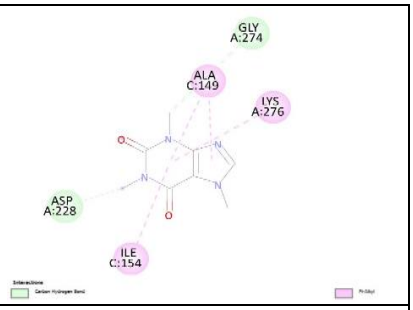
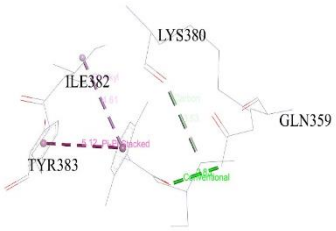
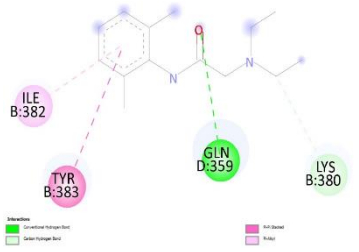
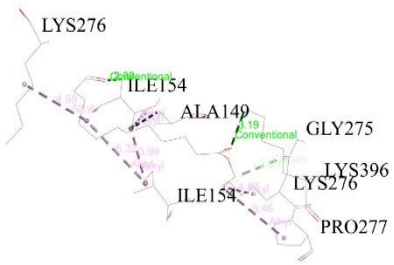
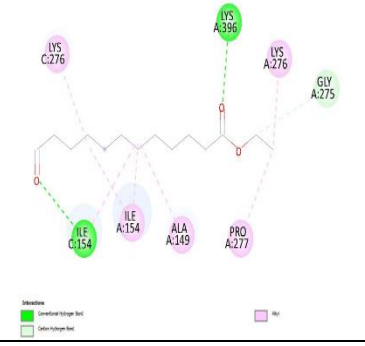
1.	21674156	-7.2		
2.	11005	-6.8		
3.	16066	-6.8		
4.	6781	-5.9		

5.	2519	-5.8		
6.	3676	-5.4		
7.	560304	-5.4		

Molecular dynamics simulation

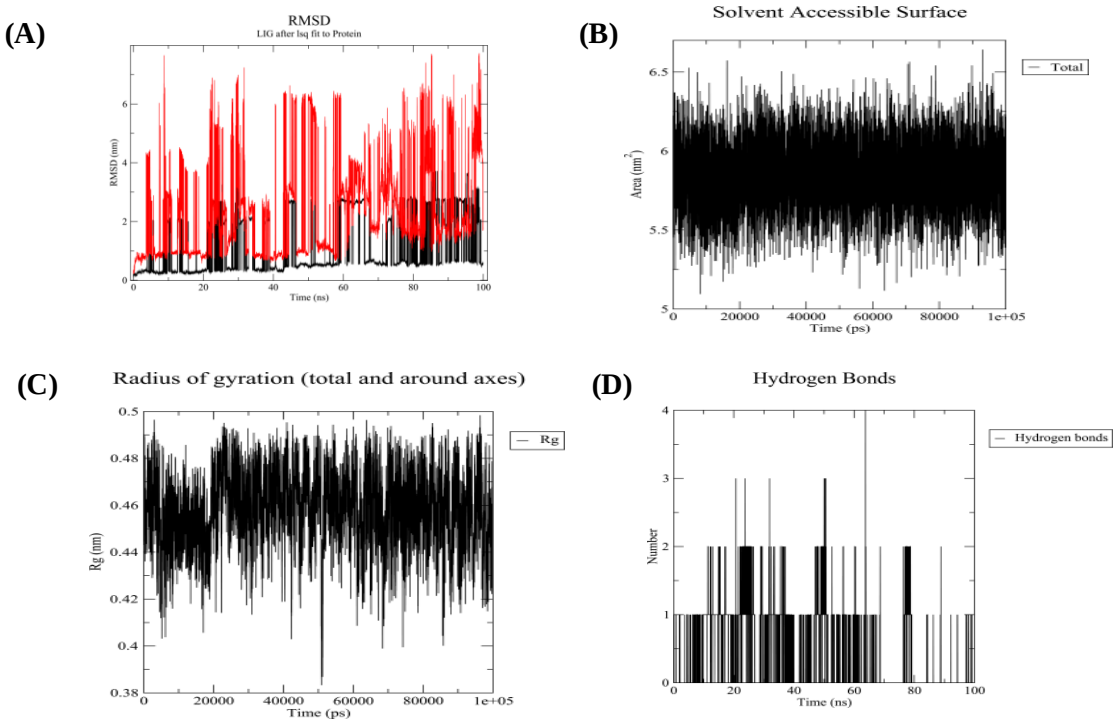


Figure 2: Molecular dynamic simulation of 1, 4-Dibutyl benzene-1,4-dicarboxylate with Tumor protein P53.

Figure-2 MD simulation trajectory plots of Tumor protein P53-1,4-Dibutylbenzen-1,4-dicarboxylate 100ns MD simulation (A) The RMSD of the Tumor protein P53 unbound (black) with 1,4-Dibutylbenzene-1,4-dicarboxylate (Red) (B) The solvent - accessible surface area (SASA) of molecule (c) Radius of gyration 1,4-Dibutyl benzene- 1,4-dicarboxylate (D) Plot of Hydrogen Bond Displaying the number of bond per second between Tumor protein P532-1,4-Dibutylbenzene-1,4-dicarboxylate.

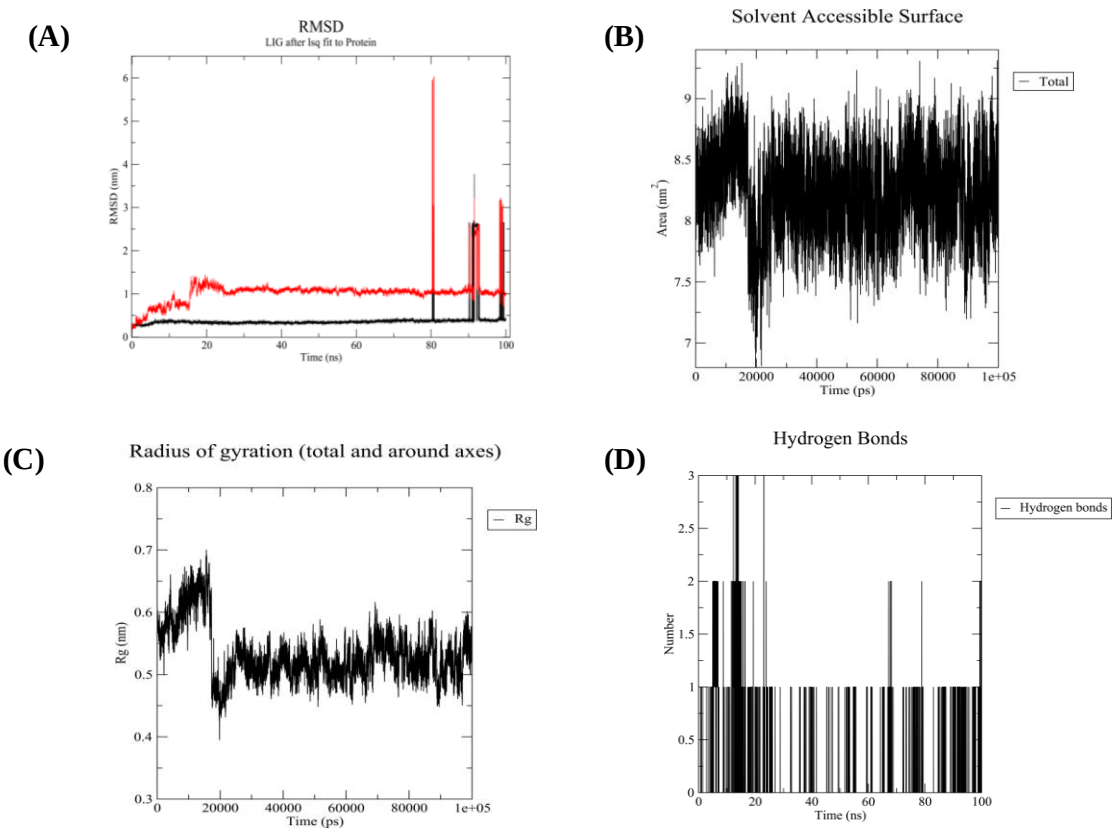


Figure 3: Molecular dynamic simulation of Alpha.-tocospiro a with KRAS (PDB ID: 4EPW).

Figure-3 MD simulation trajectory plots of Caspase 9 .alpha.-tocospiro A 100ns MD simulation (A) The RMSD of the caspase 9 unbound (black) with .alpha. - tocospiro A (Red). (B) The surface area of a molecule is accessible to solutions (SASA). (C) Radius of gyration alpha. - tocospiro A (D) Plot of Hydrogen Bond displaying the number of bonds per second between Caspase 9 .alpha.-tocospiro A

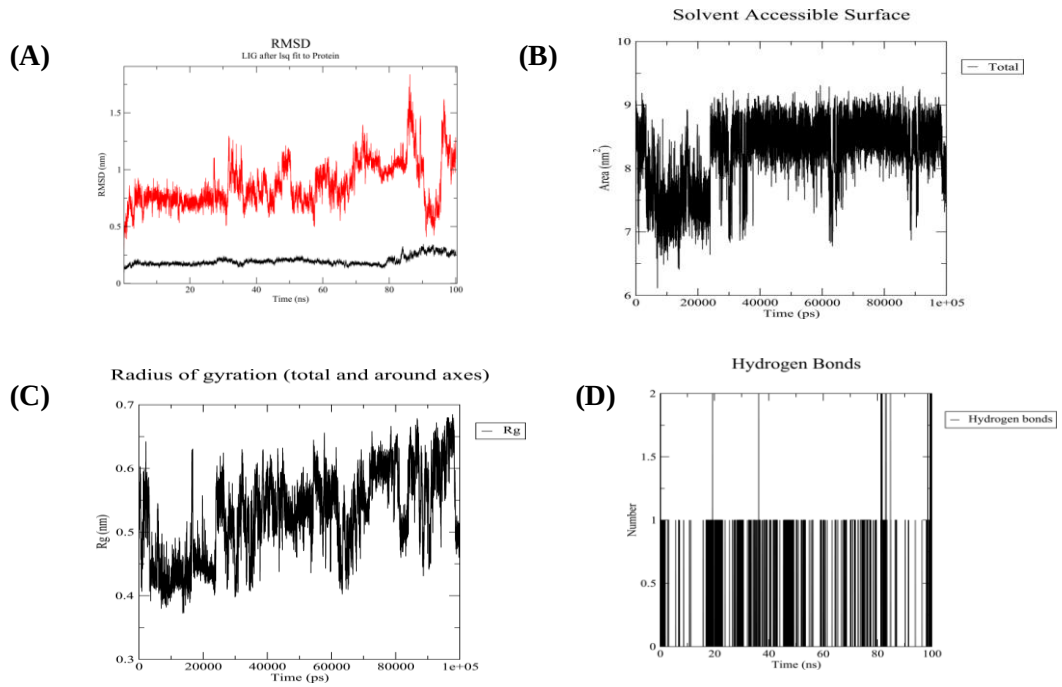


Figure 4: Molecular dynamic simulation of alpha.-tocospiro a with KRAS (PDB ID: 4EPW).

Figure-4 MD Simulation trajectory plot of KRAS- .alpha.-tocospiro A 100ns MD simulation (A) The RMSD of the KRAS unbound (black) with .Alpha.-tocospiro A (Red). (B) The surface area of the molecule is accessible to the solution (SASA). (C) Radius of gyration. alpha-tocospiro A. (D) Plot of Hydrogen Bond displaying the number of bonds per second between KRAS .alpha.-tocospiro A

DISCUSSION

The application of natural products in the search for compounds with lead based on antioxidants with anticancer effects for cancer treatment is growing. The subject of medicinal synthetic biology has a wealth of documentation regarding the use of computer algorithm approaches; nevertheless, their application to natural phytocompounds is still limited and unexplored. Molecular docking aims to help predict the complex structures of ligands and receptors by utilizing computational techniques. To align the findings according to the scores, the docking mechanism virtually identifies a library collection of compounds. Theories on the ligands' structural inhibition of the target receptor which is essential for lead enhancement are developed. The factors affecting docking include its hydrophobicity bipolarity, electrostatic attraction, hydrogen bonds, and bond width, angle, and dihedral angle of the intermolecular interactions. We used molecular docking to examine the interactions between *Croton bonplandianum* phytocompounds (1,4-Benzenedicarboxylic acid, diethyl ester, Tetradecanoic

acid, caffeine, Lidocaine, 1,4-Dibutyl benzene-1,4-dicarboxylate, 12-Oxododecanoic acid, ethyl ester, Alpha.-toco spiro A) and tumor proteins P53, Caspase 9, and KRAS. These compounds are selected based on the following Lipinski rule of five, high gastrointestinal absorption and no carcinogenicity, mutagenicity, and cytotoxicity. To describe the compounds' chemical features, Lipinski came up with the "Rule of 5" (Ro5). The rule is useful while developing new medications. The phytocompounds examined in this study were determined to be completely within Lipinski's limit range, according to the results. To find compounds with a good chance of being drug candidates, Ro5 was utilized as a tool. The substances extracted from Croton bonplandianum have the potential to be well absorbed in the intestines based on their gastrointestinal absorption. When taken orally, it is claimed that such compounds are accessible from the intestinal tract. Among the many signaling pathways involved, the human gene TP53 encodes tumor protein P53, a transcription factor that plays a pivotal role in tumor suppression.[5] Inducing cell cycle arrest, DNA repair, senescence, and apoptosis are only a few of p53's numerous functions as a tumor suppressor. The reported occurrence of TP53 mutations in various cancer types ranges from 10% (such as hematological malignancies) to over 100%.[6] A crucial player in the rise and spread of cancer is caspase-9 (CASP-9), an initiator caspase in the apoptosome-driven caspase cascade.[7] KRAS plays a significant role as a lung cancer biomarker. Lung cancer and other forms of epithelial cancer frequently involve the oncogenic event of KRAS mutational activation. Mutant KRAS-driven malignancies are notoriously difficult to cure, and there have been few successful attempts to create medicines that mitigate the oncogenic effects of this gene.[8]

CONCLUSION

According to the research's findings, plant compounds from Croton bonplandianum may be useful in the creation of modern therapies for lung cancer. To confirm these results and investigate the possible therapeutic uses of these substances, more investigation and clinical studies are necessary. The research highlights the potential of natural phytocompounds as valuable sources for the discovery and development of anti-cancer agents. The identified compounds demonstrate promising binding affinity with proteins associated with lung cancer, paving the way for further exploration and development of novel therapeutic interventions for this devastating disease.

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