STM JOURNALS

Research & Reviews: Journal of
Computational Biology

https://journals.stmjournals.com/rrjocb/

ISSN: 2319-3433 (Online)
ISSN: 2349-3720 (Print)
Volume 13, Issue 1, 2024
January–April
DOI (Journal): 10.37591/RRJoCB

Research RRJoCB

In Situ Investigation of *Hemidesmus indicus* Phytocompound Extracts as a Therapeutic Target for Acute Myeloid Leukemia

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Abstract

Objective: *Acute myeloid leukemia (AML) is an apoptotic condition in the hematopoietic system induced by the differentiation and maturation of stem cells into erythrocytes, and leukemic cell proliferation is suppressed as the PI3K-Akt-mTOR pathway is pharmacologically targeted with certain inhibitors. As a result, in silico molecular docking of compounds against AML receptors, CTMP, was used in computational research on Hemidesmus indicus to uncover therapeutic phytochemicals for AML. Thus, this study concludes that the identification of natural product-based therapeutic candidates can be facilitated by a combination of empirical information, in silico molecular docking, and ADME profiling.* ***Methods:*** *An in silico examination was performed to treat acute myeloid leukemia. In this study, the phytoconstituents of Hemidesmus indicus were thoroughly reviewed based on its cylindrical root as a natural remedy for ailments. The pharmacology of the selected compounds was screened for ADME drug-likeness. Ultimately, the screening process was finalized by employing PyRx and Biovia to dock the chosen compound with a leukemia-targeted suppressor protein.* ***Results:*** *Current in silico study on AML, Quercetin, the most abundant flavonoids with multiple properties capable of reducing cell growth in cancer cells with acute myeloid leukemic condition.* ***Conclusion:*** *Overall, the notable insights in pharmacological research studies on AML at the level of its immature stem cells and a better understanding of the molecular mechanism of the disorder would be beneficial to recommend H. indicus root as a therapeutic target. AML-related proteins were retrieved from the PDB database for molecular docking results in the discovery of a new drug and medicinal therapeutic agent for the most effective root-based H. indicus. The CTMP protein acts as an anti-leukemic suppressor protein, which develops effective protein-ligand complexes.*

Keywords: *Hemidesmus indicus*, acute myeloid leukemia, phytocompounds, pharmacology, apoptosis, therapeutic targets

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Received Date: March 27 2024
Accepted Date: April 03, 2024
Published Date: April 13, 2024

Citation: Roshithaa Pillai. In Situ Investigation of *Hemidesmus indicus* Phytocompound Extracts as a Therapeutic Target for Acute Myeloid Leukemia. Research & Reviews: Journal of Computational Biology. 2024; 13(1): 38–53p.

INTRODUCTION

Anemia of myeloid lineage stem cells (platelets, red blood cells, and white blood cells other than B and T cells) is known as acute myeloid leukemia (AML) [1]. Fever, weakness, exhaustion, appetite loss, weight loss, and joint or bone pain are early indicators of AML [2]. Small red patches on the skin, easy bruising, bleeding, recurrent mild infections, and slow wound healing are all indicative of AML. Similar to other cancers, neoplastic alterations and clonal expansion are

caused by genetic differences [3, 4]. Research indicates that individuals with AML who receive consolidation treatment have better overall survival rates 3–5. Maintenance therapy is a common treatment option [5]. Its significance in AML is still debated, although it has helped bring about long-lasting, durable remission and cure in other hematological malignancies, such as follicular lymphoma and acute lymphoblastic leukemia [6]. To best customize AML therapy in the current era, genetic and molecular profiling is crucial, as evidenced by the FDA approval of small-molecule inhibitors for AML subgroups [7].

Integrating anemia with its therapeutic medicinal plant, *Hemidesmus indicus*, a climbing plant of the Apocynaceae family, is commonly referred to as the "Indian Sarsaparilla" and is found in the central, western, and southern regions of India. Cylindrical root with corky bark and dark brown with wrinkles. The aromatic roots of *H. indicus* are mostly bioactive in the form of 2-hydroxy-4-methoxybenzaldehyde. With many pharmacological activities, such as antibacterial, anti-enterobacterial, anti-acne, antioxidant, hepatoprotective, and anti-inflammatory properties, the root is the most beneficial portion of *H. indicus* [8]. *H. indicus* root's cytodifferentiation, cytostatic, and antileukemic properties of *H. indicus* root in the root bark have been supported by several laboratory investigations as anticancer drugs [9]. As the roots of plants contain bioactive substances, such as tannins, phenols, saponins, flavonoids, coumarins, and terpenoids, its extract has been shown to protect cultured lymphocytes against genotoxicity [8].

Human carboxyl-terminal modulator protein (CTMP) or thioesterase superfamily member 4 (THEM4) is an endogenous inhibitor of protein kinase B (Akt), which may attach to Akt and dephosphorylate it to prevent downstream signal transmission. THEM4 has anti-inflammatory and antitumor actions because it reversely controls phosphatidylinositol 3-kinase (PI3K)/Akt [10]. The polyherbal formulation also showed high in vitro cytotoxicity, anti-inflammatory properties, and controlled apoptosis in human hepatocellular cancer. According to previous research, this protein is the optimal target molecular route for AML treatment [10]. According to early research on hTHEM4 activity, it interacts with membrane-bound Akt1 to prevent upstream protein kinases from activating it. One well-known mechanism for preventing apoptosis is activated Akt1. The process by which hTHEM4 sensitizes cells to apoptosis has been shown in a recent study [10].

This study aimed to aid MD stimulation and future in vivo experiments investigating quercetin, a pharmacological component derived from *H. indicus* root, may be used as a potential therapeutic target.

METHODOLOGY

Extraction and Purification of Target Protein

Human carboxyl-terminal modulator protein [PDBID: 4GAH] data were retrieved from the PDB database (*https://www.rcsb.org/*) at a resolution of 2.30 Å. Proteins were extracted using X-ray diffraction. It is a protein macromolecular structure with 3,250 atoms and 367 modeled residues, with a structural molecular weight of 50.74 Da [11].

The chain of this protein was used for the analysis. Biovia Discovery Studio (*https://discover.3ds.com/discovery-studio-visualizer-download*) was used to purify molecules. All heteroatoms and water molecules were eliminated. The chain was more defined with amino acid residues 43–98 and 106–244. Therefore, the chain and its protein groups were retained in the molecule in future studies [12].

The water molecules were completely removed during pre-docking to prevent any impact on the docking results. The refined structure was subjected to protein characterization [13].

Structure Validation

Using PDB Sum (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/[), a Ramachandran plot was generated for the refined protein, which shows the molecule(s) that make up the structure (i.e., protein chains, DNA, ligands, and metal ions) and secondary structure of the refined protein.

Extraction of Ligands

The Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) database (*https://cb.imsc.res.in/imppat/*) is a manually curated database that has been constructed via digitalization of information from books on traditional Indian medicine, published research articles, and other existing resources, providing a repository for phytochemicals of medicinal plants from India, and extracts the phytocompounds of *H. indicus* with plant part-root-based, preferring its medicinal properties.

ADME Analysis

SwissADME *(http://www.swissadme.ch/*) allows the computation of physicochemical descriptors and the prediction of ADME parameters, pharmacokinetic properties, drug-like nature, and medicinal chemistry friendliness of one or multiple small molecules to support the drug discovery of these 51 phytocompounds.

Molecular Docking

Molecular docking forecasts the main binding mode(s) of a ligand to a protein with a known three-dimensional structure. PyRx is a docking program that automates ligand purification (*https://pyrx.sourceforge.io/*). Protein active sites were identified using Biovia Discovery Studio, which involves the purification process of removing undesired heteroatoms. The desired ligands were optimized, and PyRx generated a protein macromolecule with a three-dimensional structure [14]. PyRx software was used to perform molecular docking of the optimized proteins and ligands in PDBQT format, with rigid and optimized parameters. PyRx uses its binding affinity or scoring function to predict the interaction between a ligand and protein.

RESULTS

Extraction of Target Proteins

To characterize the form, interactions, and activity of a protein, it must be extracted. During refinement, it was necessary to divide the non-protein and peptide content. Although they can influence the binding outcome and complicate the target protein, the docking process does not require attachment of water molecules or additional strands. Therefore, to clean the peptide and add polarity charges, Biovia purifies the peptide before binding. This process entails removing moisture and cleaning other chains. Figure 1 shows a visualization of the protein.

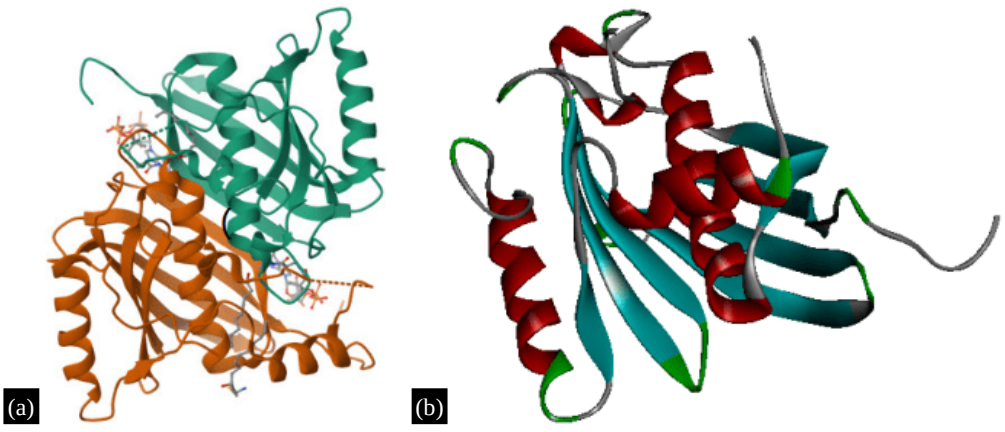


Figure 1. (a) Original protein, (b) refined proteins.

Statistical Analysis of Secondary Structure

A Ramachandran plot was used to display the descriptive statistical dispersion of the phi and psi inclinations for each peptide in the molecule. The favored regions in the Ramachandran plot were examined (Figure 2). As demonstrated by the Ramachandran plot, with an R-factor no greater than 20.0, a good-quality model would be expected to have over 90% in the most favored regions. As expected, the regions with the highest favorability contained 157 residues and encompassed 94.0% of proteins. In contrast, areas with an additional allowed region had 10 residues (6.0%), areas with a generously allowed region had 0 residues (0.0%), and areas with a disallowed region also had zero residues (0.0%). The end residues, excluding Proline and Glycine, were 4. Proline and Glycine residues were found in the order 11 and 13, respectively.

As illustrated in Figure 3, PDBsum provided the secondary structure of the refined protein. It comprises one sheet, four β-hairpins, five β-bulges, seven strands, five helices, and 15 β-turns.

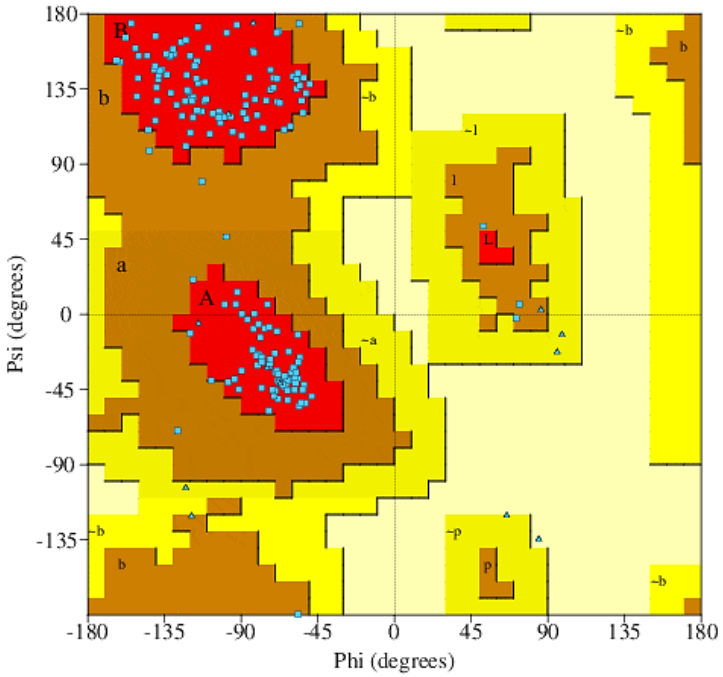


Figure 2. Ramachandran plot for refined proteins.

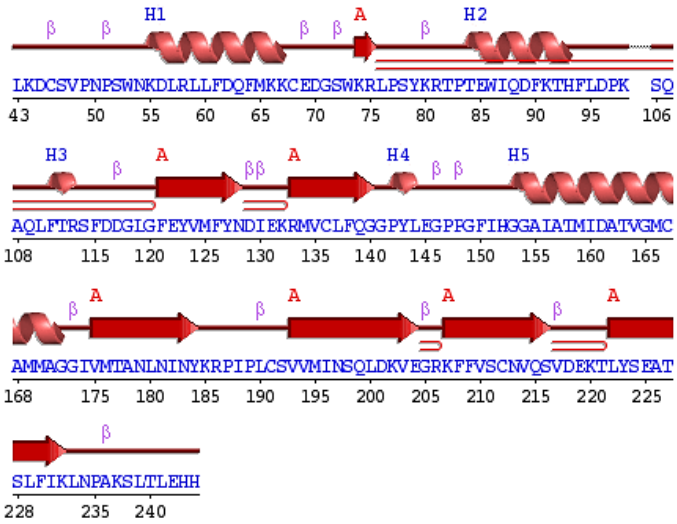


Figure 3. Secondary structure of the refined protein.

Extraction of Ligands

The phytocompounds of *H. indicus* were isolated from their roots. The canonical sequences were retrieved from the PubChem database (Table 1). The significance of therapeutic chemicals in biological systems lies in their ability to remove nondrug-like candidates from a pool of bioactive compounds, which is crucial for contemporary drug development and discovery.

Table 1. Screening of phytocompounds of *H. indicus* plant-root based on corresponding smiles.

S.N.	Ligand Name	Canonical Smiles
1.	Myrtenol	OCC1=CCC2CC1C2(C)C
2.	Syringic acid	COc1cc(cc(c1O)OC)C(=O)O
3.	Vanillin	COc1cc(C=O)ccc1O
4.	Pinocarvone	C=C1C(=O)CC2CC1C2(C)C
5.	Isovanillin	O=Cc1ccc(c(c1)O)OC
6.	Lauric acid	CCCCCCCCCCCC(=O)O
7.	Methyl salicylate	COC(=O)c1ccccc1O
8.	Decanoic acid	CCCCCCCCCC(=O)O
9.	2,5-Dihydroxybenzoic acid	Oc1ccc(c(c1)C(=O)O)O
10.	Octanoic acid	CCCCCCCC(=O)O
11.	4-Methoxybenzaldehyde	COc1ccc(cc1)C=O
12.	Benzophenone	O=C(c1ccccc1)c1ccccc1
13.	Dihydrocarvyl acetate	CC(=O)OC1CC(CCC1C)C(=C)C
14.	Verbenone	CC1=CC(=O)C2CC1C2(C)C
15.	Quercetin	Oc1cc(O)c2c(c1)oc(c(c2=O)O)c1ccc(c(c1)O)O
16.	4-Methoxysalicylic acid	COc1ccc(c(c1)O)C(=O)O
17.	Thymol	Cc1ccc(c(c1)O)C(C)C
18.	2-Hydroxy-4-methoxybenzaldehyde	COc1ccc(c(c1)O)C=O
19.	Salicylaldehyde	O=Cc1ccccc1O
20.	Hydroquinone	Oc1ccc(cc1)O
21.	Linalyl acetate	C=CC(OC(=O)C)(CCC=C(C)C)C
22.	Vanillic acid	COc1cc(ccc1O)C(=O)O
23.	Palmitic acid	CCCCCCCCCCCCCCCC(=O)O
24.	Hexatriacontane	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
25.	2,10-Epoxypinane	CC1(C)C2CCC3(C1C2)CO3
26.	Eucalyptol	CC12CCC(CC1)C(O2)(C)C
27.	beta-Elemene	C=C[C@]1(C)CC[C@H](C[C@H]1C(=C)C)C(=C)C
28.	Benzyl benzoate	O=C(c1ccccc1)OCc1ccccc1
29.	3-Methoxyphenol	COc1cccc(c1)O
30.	Phenethyl cinnamate	O=C(/C=C/c1ccccc1)OCCc1ccccc1
31.	Pentyl cinnamate	CCCCCOC(=O)/C=C/c1ccccc1
32.	4-Carvomenthenol	CC1=CCC(CC1)(O)C(C)C
33.	Guaiacol	COC1=CC=CC=C1O
34.	Alpha-Terpinyl acetate	CC(=O)OC(C1CCC(=CC1)C)(C)C
35.	Ferulic acid	COc1cc(/C=C/C(=O)O)ccc1O
36.	3,4-Dihydroxybenzoic acid	OC(=O)c1ccc(c(c1)O)O
37.	Caffeic acid	OC(=O)/C=C/c1ccc(c(c1)O)O
38.	Cinnamic acid	OC(=O)/C=C/c1ccccc1
39.	4-Hydroxycinnamic acid	OC(=O)/C=C/c1ccc(cc1)O
40.	Gallic acid	OC(=O)c1cc(O)c(c(c1)O)O
41.	Camphor	O=C1CC2C(C1(C)CC2)(C)C

S.N.	Ligand Name	Canonical Smiles
42.	alpha-Terpineol	CC1=CCC(CC1)C(O)(C)C
43.	Levomenol	CC(=CCC[C@@]([C@H]1CCC(=CC1)C)(O)C)C
44.	Isocaryophyllene	C/C/1=C/CCC(=C)[C@@H]2[C@@H](CC1)C(C2)(C)C
45.	beta-Selinene	C=C1CCC[C@]2([C@H]1C[C@@H](CC2)C(=C)C)C
46.	Aromadendrene	CC1CCC2C1C1C(C1(C)C)CCC2=C
47.	Bornyl acetate	CC(=O)OC1CC2C(C1(C)CC2)(C)C
48.	Ledol	C[C@@H]1CC[C@H]2[C@@H]1[C@H]1[C@H](C1(C)C)CC[C@@]2(C)O
49.	Limonene	CC1=CCC(CC1)C(=C)C
50.	Alpha-Muurolol	CC1=C[C@@H]2[C@H](CC1)[C@](C)(O)CC[C@@H]2C(C)C
51.	Nerolidol	C=CC(CC/C=C(/CCC=C(C)C)C)(O)C

ADME Analysis

The fate of putative drug candidates in biological systems is dictated by their absorption (A), distribution (D), metabolism (M), and excretion. ADME analysis of the chosen phytocompounds was performed using SwissADME. The ADME analysis screening process has attributes that are sufficiently developed to be classified as consumable bioactive compounds; the list is dropped into Table 2. Pharmacokinetics assesses the effects of the medication in the biological system by examining HIA, BBB permeant, P-GP substrate, and LOG KP, which are indicated in Table 3. According to Table 4, which summarizes the biological characteristics governing ligand selection for further docking processes, drug-likeness is a crucial component of drug development.

The delimitation of the molecular structure of the phytocompounds was reinterpreted as canonical smiles that were unique identifications of ligands of *H. indicus* root-based plant being extracted and interpreted in Table 1. The pharmacokinetic parameters of the 51 potential chirals were predicted using the physicochemical characteristics of the phytocompounds. The molecular weight (MW), range of hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and rotatable bonds (RA) with regard to increased RA numbers represent the stability of bioactive substances. Topological polar surface area (TPSA); no. for heavy atoms(HA), and no. of aromatic heavy atoms (AHA), fraction Csp3, and molar reactivity (MR), were evaluated for potential bioactive phytocompounds, as shown in Table 2.

Table 2. Physiochemical properties for the phytocompounds of *H. indicus.*

S.N.	Molecule name	Formula	MW	HA	AHA	Fraction CSP3	RB	HBA	HBD	MR	TPSA
1.	Myrtenol	C10H16O	152.23	11	0	0.80	1	1	1	46.38	20.23
2.	Syringic acid	C9H10O5	198.17	14	6	0.22	3	5	2	48.41	75.99
3.	Vanillin	C8H8O3	152.15	11	6	0.12	2	3	1	40.34	46.53
4.	Pinocarvone	C10H14O	150.22	11	0	0.70	0	1	0	45.42	17.07
5.	Isovanillin	C8H8O3	152.15	11	6	0.12	2	3	1	40.34	46.53
6.	Lauric acid	C12H24O2	200.32	14	0	0.92	10	2	1	61.57	37.30
7.	Methyl salicylate	C8H8O3	152.15	11	6	0.12	2	3	1	39.74	46.53
8.	Decanoic acid	C10H20O2	172.26	12	0	0.90	8	2	1	51.96	37.30
9.	2,5-Dihydroxybenzoic acid	C7H6O4	154.12	11	6	0	1	4	3	37.45	77.76
10.	Octanoic acid	C8H16O2	144.21	10	0	0.88	6	2	1	42.34	37.30
11.	4-Methoxybenz aldehyde	C8H8O2	136.15	10	6	0.12	2	2	0	38.32	26.30
12.	Benzophenone	C13H10O	182.22	14	12	0	2	1	0	56.32	17.07
13.	Dihydrocarvyl acetate	C12H20O2	196.29	14	0	0.75	3	2	0	58.49	26.30
14.	Verbenone	C10H14O	150.22	11	0	0.70	0	1	0	45.42	17.07

S.N.	Molecule name	Formula	MW	HA	AHA	Fraction CSP3	RB	HBA	HBD	MR	TPSA
15	Quercetin	C15H10O7	302.24	22	16	0	1	7	5	78.03	131.36
16	4-Methoxysalicylic acid	C8H8O4	168.15	12	6	0.12	2	4	2	41.92	66.76
17.	Thymol	C10H14O	150.22	11	6	0.40	1	1	1	48.01	20.23
18.	2-Hydroxy-4-methoxybenzaldehyde	C8H8O3	152.15	11	6	0.12	2	3	1	40.34	46.53
19.	Salicylaldehyde	C7H6O2	122.12	9	6	0	1	2	1	33.85	37.30
20.	Hydroquinone	C6H6O2	110.11	8	6	0	0	2	2	30.49	40.46
21.	Linalyl acetate	C12H20O2	196.29	14	0	0.58	6	2	0	60.17	26.30
22.	Vanillic acid	C8H8O4	168.15	12	6	0.12	2	4	2	41.92	66.76
23.	Palmitic acid	C16H32O2	256.42	18	0	0.94	14	2	1	80.80	37.30
24.	Hexatriacontane	C36H74	506.97	36	0	1.00	33	0	0	175.1	0
25.	2,10-Epoxypinane	C10H16O	152.23	11	0	1	0	1	0	44.7	12.53
26.	Eucalyptol	C10H18O	154.25	11	0	1	0	1	0	47.12	9.23
27.	beta-Elemene	C15H24	204.35	15	0	0.60	3	0	0	70.42	0
28.	Benzyl benzoate	C14H12O2	212.24	16	12	0.07	4	2	0	62.21	26.30
29.	3-Methoxyphenol	C7H8O2	124.14	9	6	0.14	1	2	1	34.96	29.46
30.	Phenethyl cinnamate	C17H16O2	252.31	19	12	0.12	6	2	0	76.73	26.30
31.	Pentyl cinnamate	C14H18O2	218.29	16	6	0.36	7	2	0	66.66	26.30
32.	4-Carvomenthenol	C10H18O	154.25	11	0	0.80	1	1	1	48.80	20.23
33.	Guaiacol	C7H8O2	124.14	9	6	0.14	1	2	1	34.96	29.46
34.	alpha-Terpinyl acetate	C12H20O2	196.29	14	0	0.75	3	2	0	58.53	26.30
35	Ferulic acid	C10H10O	194.18	14	6	0.10	3	4	2	51.63	66.76
36.	3,4-Dihydroxybenzoic acid	C7H6O4	154.12	11	6	0	1	4	3	37.45	77.76
37.	Caffeic acid	C9H8O4	180.16	13	6	0	2	4	3	47.16	77.76
38.	Cinnamic acid	C9H8O2	148.16	11	6	0	2	2	1	43.11	37.30
39.	4-Hydroxy cinnamic acid	C9H8O3	164.16	12	6	0	2	3	2	45.13	57.53
40.	Gallic acid	C7H6O5	170.12	12	6	0	1	5	4	39.47	97.99
41.	Camphor	C10H16O	152.23	11	0	0.90	0	1	0	45.64	17.07
42.	alpha-Terpineol	C10H18O	154.25	11	0	0.80	1	1	1	48.80	20.23
43.	Levomenol	C15H26O	222.37	16	0	0.73	4	1	1	72.36	20.23
44.	Isocaryophyllene	C15H24	204.35	15	0	0.73	0	0	0	68.78	0
45.	beta-Selinene	C15H24	204.35	15	0	0.73	1	0	0	68.78	0
46.	Aromadendrene	C15H24	204.35	15	0	0.87	0	0	0	67.14	0
47.	Bornyl acetate	C12H20O2	196.29	14	0	0.92	2	2	0	56.33	26.30
48.	Ledol	C15H26O	222.37	16	0	1	0	1	1	68.82	20.23
49.	Limonene	C10H16	136.23	10	0	0.60	1	0	0	47.12	0
50.	alpha-Muurolol	C15H26O	222.37	16	0	0.87	1	1	1	70.72	20.23
51.	Nerolidol	C15H26O	222.37	16	0	0.60	7	1	1	74.0	20.23

For the ADME characteristics, all phytoconstituents were assessed and are listed in Table 3. Of the 51 phytochemicals, 45 were screened based on high Human Intestinal Absorption (HIA) with optimal solubility. For tissues and organs to use high HIA, oral administration would instantly absorb compounds, and compounds with limited HIA absorption could be administered through other routes that must be absorbed into the bloodstream. This allows medications to be made bioavailable to the human body. The substratum for permeability glycoproteins (P-GP) often mediates the emergence of resistance to anticancer medications and is responsible for reducing drug accumulation in multidrug-resistant cells. LOGKp (Skin permeation) facilitates the cutaneous absorption of medication.

Table 3. Pharmacokinetics extracted from *H. indicus.*

S.N.	Molecule Name	GI Absorption	BBB Permeant	P-GP Substrate	LOG KP
1.	Myrtenol	high	yes	no	-4.94
2.	Syringic acid	high	no	no	-6.77
3.	Vanillin	high	yes	no	-6.37
4.	Pinocarvone	high	yes	no	-5.68
5.	Isovanillin	high	yes	no	-6.54
6.	Lauric acid	high	yes	no	-4.54
7.	Methyl salicylate	high	yes	no	-5.42
8.	Decanoic acid	high	yes	no	-4.45
9.	2,5-Dihydroxybenzoic acid	high	no	no	-6.00
10.	Octanoic acid	high	yes	no	-5.01
11.	4-Methoxybenzaldehyde	high	yes	no	-5.88
12.	Benzophenone	high	yes	no	-5.15
13.	Dihydrocarvyl acetate	high	yes	no	-4.81
14.	Verbenone	high	yes	no	-5.63
15.	Quercetin	high	no	no	-7.05
16.	4-Methoxysalicylic acid	high	no	no	-5.93
17.	Thymol	high	no	no	-4.87
18.	2-Hydroxy-4-methoxybenzaldehyde	high	yes	no	-6.61
19.	Salicylaldehyde	high	yes	no	-5.76
20.	Hydroquinone	high	yes	no	-6.55
21.	Linalyl acetate	high	yes	no	-4.71
22.	Vanillic acid	high	no	no	-6.31
23.	Palmitic acid	high	yes	no	-2.77
24.	Hexatriacontane	low	no	yes	4.18
25.	2,10-Epoxypinane	high	yes	no	-5.77
26.	Eucalyptol	high	yes	no	-5.30
27.	beta-Elemene	low	no	No	-3.21
28.	Benzyl benzoate	high	Yes	no	-4.78
29.	3-Methoxyphenol	high	yes	no	-6.11
30.	Phenethyl cinnamate	high	yes	no	-4.59
31.	Pentyl cinnamate	high	yes	no	-4.49
32.	4-Carvomenthenol	high	yes	no	-4.93
33.	Guaiacol	high	yes	no	-6.12
34.	alpha-Terpinyl acetate	high	yes	no	-4.69
35.	Ferulic acid	high	yes	no	-6.41
36.	3,4-Dihydroxybenzoic acid	high	no	no	-6.42
37.	Caffeic acid	high	no	no	-6.58
38.	Cinnamic acid	high	yes	no	-5.69
39.	4-Hydroxycinnamic acid	High	yes	no	-6.26
40.	Gallic acid	high	no	no	-6.84
41.	Camphor	high	yes	No	-5.67
42.	alpha-Terpineol	high	yes	No	-4.83
43.	Levomenol	high	yes	no	-4.97
44.	Isocaryophyllene	low	no	no	-4.44
45.	beta-Selinene	low	no	no	-3.68
46.	Aromadendrene	low	yes	no	-4.20
47.	Bornyl acetate	high	yes	no	-4.44

S.N.	Molecule Name	GI Absorption	BBB Permeant	P-GP Substrate	LOG KP
48.	Ledol	high	yes	no	-5.00
49.	Limonene	low	yes	no	-3.89
50.	alpha-Muurolol	high	yes	no	-5.29
51.	Nerolidol	high	yes	no	-4.23

If a medication or substance does not experience a violation, Lipinski said it would be a potential option for preclinical testing. The acceptable MW range must not exceed 500 Da. The permissible range for the HBA must be less than or equal to 10. The compound's HBD must fall within the permitted range of less than or equal to 5. The Log P value should not exceed 5. Ghose, Veber, Egan, and Muegge's rules were used to screen the ligands. Of the 51 compounds evaluated, 45 were shortlisted for further studies, as illustrated in Table 4.

Table 4. Drug likeness for the phytocompounds extracted from *Hemidesmus indicus.*

S.N.	Molecule Name	LIPINSKI	GHOSE	VEBER	EGAN	MUEGGE	BIO Availability
1.	Myrtenol	yes	no	yes	yes	no	0.55
2.	Syringic acid	yes	yes	yes	yes	no	0.56
3.	Vanillin	yes	no	yes	yes	no	0.55
4.	Pinocarvone	yes	no	yes	yes	no	0.55
5.	Isovanillin	yes	no	yes	yes	no	0.55
6.	Lauric acid	yes	yes	yes	yes	yes	0.85
7.	Methyl salicylate	yes	no	yes	yes	no	0.55
8.	Decanoic acid	yes	yes	yes	yes	no	0.85
9.	2,5-Dihydroxybenzoic acid	Yes	no	yes	yes	no	0.56
10.	Octanoic acid	yes	no	yes	yes	no	0.85
11.	4-Methoxybenzaldehyde	yes	no	yes	yes	no	0.55
12.	Benzophenone	yes	yes	yes	yes	no	0.55
13.	Dihydrocarvyl acetate	yes	yes	yes	yes	no	0.55
14.	Verbenone	yes	no	yes	yes	no	0.55
15.	Quercetin	Yes	Yes	yes	yes	yes	0.55
16.	4-Methoxysalicylic acid	yes	yes	yes	yes	no	0.85
17.	Thymol	yes	no	Yes	yes	no	0.55
18.	2-Hydroxy-4-methoxybenzaldehyde	yes	no	yes	yes	no	0.55
19.	Salicylaldehyde	yes	no	yes	yes	no	0.55
20.	Hydroquinone	yes	no	yes	yes	no	0.55
21.	Linalyl acetate	yes	yes	yes	yes	no	0.55
22.	Vanillic acid	yes	yes	yes	yes	no	0.85
23.	Palmitic acid	no	yes	no	yes	no	0.85
24.	Hexatriacontane	no	no	no	no	no	0.17
25.	2,10-Epoxypinane	yes	no	yes	yes	no	0.55
26.	Eucalyptol	yes	no	yes	yes	no	0.55
27.	beta-Elemene	no	yes	yes	yes	no	0.55
28.	Benzyl benzoate	yes	yes	Yes	yes	yes	0.55
29.	3-Methoxyphenol	yes	no	yes	yes	no	0.55
30.	Phenethyl cinnamate	yes	yes	yes	yes	yes	0.55
31.	Pentyl cinnamate	Yes	yes	yes	yes	yes	0.55
32.	4-Carvomenthenol	yes	no	yes	yes	no	0.55
33.	Guaiacol	yes	no	yes	yes	no	0.55

S.N.	Molecule Name	LIPINSKI	GHOSE	VEBER	EGAN	MUEGGE	BIO Availability
34.	alpha-Terpinyl acetate	yes	yes	yes	yes	no	0.55
35.	Ferulic acid	yes	Yes	yes	yes	no	0.85
36.	3,4-Dihydroxybenzoic acid	yes	no	yes	yes	no	0.56
37.	Caffeic acid	yes	yes	yes	yes	no	0.56
38.	Cinnamic acid	yes	no	yes	yes	no	0.85
39.	4-Hydroxycinnamic acid	yes	yes	yes	yes	no	0.85
40.	Gallic acid	yes	no	yes	yes	no	0.56
41.	Camphor	yes	no	yes	yes	no	0.55
42.	alpha-Terpineol	yes	no	yes	yes	no	0.55
43.	Levomenol	Yes	yes	yes	yes	no	0.55
44.	Isocaryophyllene	no	yes	yes	yes	no	0.55
45.	beta-Selinene	no	yes	yes	yes	no	0.55
46.	Aromadendrene	no	yes	yes	Yes	no	0.55
47.	Bornyl acetate	yes	yes	yes	yes	no	0.55
48.	Ledol	yes	yes	yes	yes	no	0.55
49.	Limonene	yes	no	yes	yes	no	0.55
50.	alpha-Muurolol	yes	yes	yes	yes	no	0.55
5151.	Nerolidol	yes	yes	yes	yes	no	0.55

Boiled Egg Analysis

The ability of substances to have high HIA and BBB permeants was assessed using the central nervous system or gastrointestinal estimated permeability prediction model. Therefore, in the Cartesian plane, the likelihood of the BBB is increased if the physicochemical space of molecules rests in the yolk region, which is represented by the yellow ellipse; conversely, the conjecture of HIA is increased if the physicochemical space of molecules rests in the albumin region (white areas). In addition, Beta-Elemene lies in gray areas outside the graph's range and excludes the “egg,” claiming that the chemicals are neither absorbent nor brain-piercing, which is why they are regarded as a noted box. There was no conflict of interest between the albumin and yolk regions. PGP+ (red circle) indicates the presence of PGP-(blue circle), indicating the absence of the compounds (Figure 4).

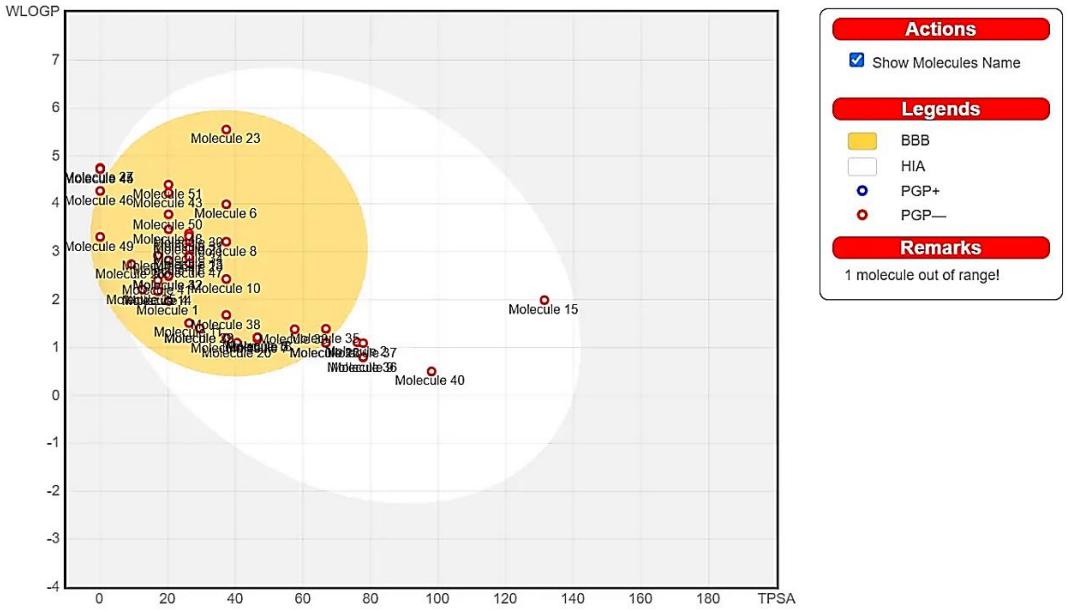


Figure 4. Boiled-egg analysis.

MOLECULAR DOCKING AND VISUALIZATION

The molecular dynamic simulation was initiated by molecular docking, which was utilized to determine the binding energy of the protein-ligand interaction. The optimal molecular docking poses were analyzed and visualized using forty-five optimized molecules. The docking process was validated prior to ligand screening. Before performing a docking study, each protein structure was refined and its energy was optimized. A protein-ligand complex with a known three-dimensional structure can have its binding affinity calculated using the Dock Score, also known as the binding affinity score. The ligands in this study that bind similarly to the reference ligands were identified using the virtual screening technique (Table 5).

Table 5. The binding affinity of the refined protein 4GAH and ligands from *H. indicus*.

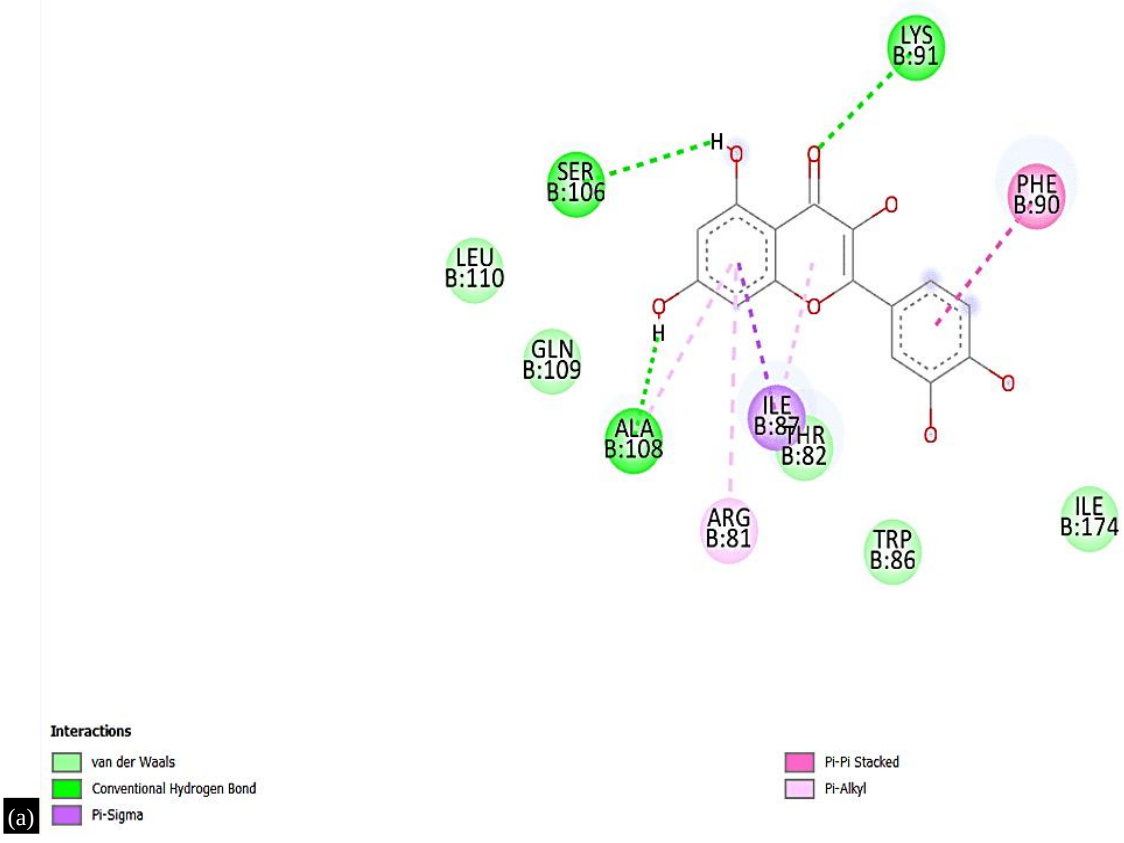
Protein-Ligand Name	Binding Affinity
4GAH_Myrtenol	-5.4
4GAH_Syringic acid	-5.3
4GAH_Alpha-Terpinyl acetate	-5.8
4GAH_4-terpineol	-5.5
4GAH_Vanillin	-5.1
4GAH_Isovanillin	-4.9
4GAH_Pinocarvone	-5.6
4GAH_Alpha-terpineol	-5.8
4GAH_Dipentene	-5.9
4GAH_Benzyl benzoate	-6.3
4GAH_Camphor	-5.3
4GAH_Eucalyptol	-5.3
4GAH_Verbenone	-5.8
4GAH_Decanoic acid	-4.9
4GAH_Dihydrocarveol acetate	-6.1
4GAH_Benzophenone	-6.8
4GAH_P-methoxybenzaldehyde	-4.6
4GAH_2,5-dihydroxybenzoic acid	-4.9
4GAH_Gallic acid	-5.1
4GAH_Octanoic acid	-4.5
4GAH_Dodecanoic acid	-4.7
4GAH_Methyl salicylate	-5.1
4GAH_Levomenol	-6.5
4GAH_Cinnamic acid	-5.6
4GAH_Phenethyl cinnamate	-6.6
4GAH_4-Hydroxycinnamic acid	-5.4
4GAH_Bornyl acetate	-5.8
4GAH_Caffeic Acid	-5.8
4GAH_2-Hydroxy-4-methoxy benzaldehyde	-4.7
4GAH_Thymol	-6.0
4GAH_Salicylaldehyde	-4.6
4GAH_3,4-Dihydroxybenzoic acid	-5.9
4GAH_4-Methoxysalicylic acid	-5.0
4GAH_Hydroquinone	-4.3
4GAH_Linalyl acetate	-5.2
4GAH_Vanillic acid	-4.8
4GAH_3-Methoxyphenol	-4.7
4GAH_2,10-Epoxypinane	-5.5

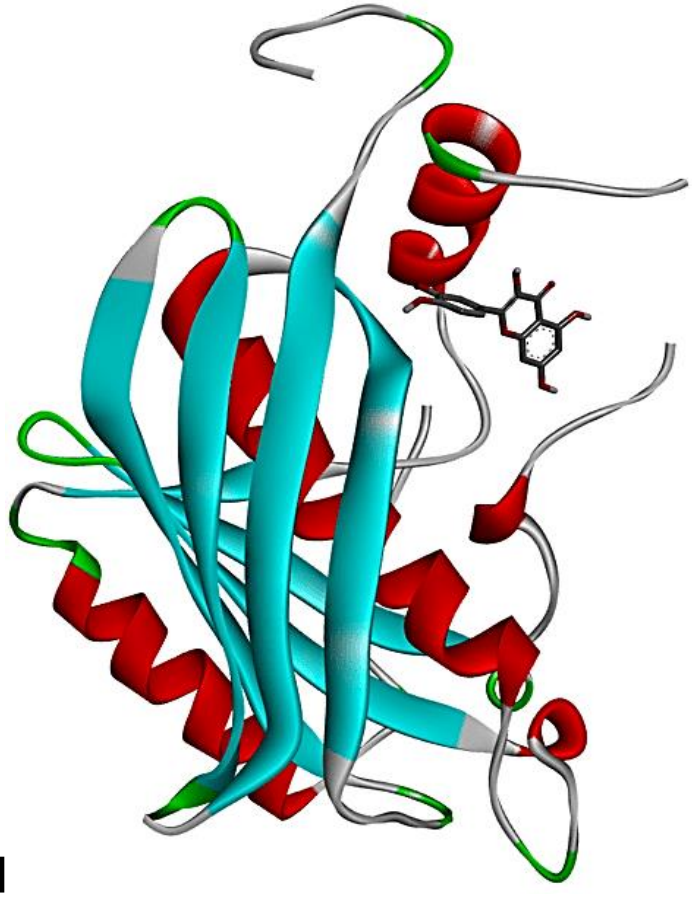
Protein-Ligand Name	Binding Affinity
4GAH_Quercetin	-7.1
4GAH_Pentyl cinnamate	-5.8
4GAH_Guaiacol	-4.9
4GAH_Ferulic acid	-5.5
4GAH_Ledol	-6.7
4GAH_alpha-Muurolol	-6.3
4GAH_Nerolidol	-6.2

The following 45 compounds were docked. The highest negative binding ligand, quercetin, had a-7.1 affinity extracted from *H. indicus* root part and was selected for visualization of the protein-ligand complex, which can be used for further docking studies (Figure 5).

Interactions between the target receptors and putative inhibitors. Similar linkages have been implicated; however, typical inhibitors and prospective inhibitors have distinct amino acids in their binding locations. Interacting bonds include salt bridges, hydrogen bonding, van der Waals forces, and other types of π-bonds. Hydrogen-bond-based binding interactions are more robust. The primary mechanism behind cell-to-cell communication is often the unique chemical interactions that occur between ligands and receptors. Disorders involving this dysregulation have been linked to cancer, autoimmune disorders, and neurodegenerative diseases.

Figure 5 shows the 2D and 3D interactions of the ligand with the protein. Numerous types of amino acids were observed in the 2D structure: (I)Lysine, Serine, and alanine with conventional hydrogen bonds; (II) leucine, glutamine, threonine, tryptophan, and isoleucine with van der Waals interactions; (III) arginine with Pi-alkyl bonds; (IV) Isoleucine with Pi-Sigma bond; and (V) Phenylalanine with Pi-Pi stalked bonds. Hydrophobic attractions were also observed for these amino acids.





(b)

Figure 5. (a) Best 2D ligand-interaction (b)3D structure of the protein-ligand complex.

DISCUSSION

CTMP overexpression causes cytochrome c release from the mitochondria into the cytosol and decreases membrane potential. Normally, CTMP functions as an anti-leukemic suppressor. Based on these findings, CTMP may be crucial for mitochondria-mediated apoptosis. Through the release of cytochrome c from the mitochondria brought on by cell death signaling, Apaf-1, the protease activating factor-1 that triggers apoptosis, attaches to it, and causes oligomerization and the creation of apoptosome [15].

The identification of the CTMP protein, the most highly altered tumor-suppressor gene found in p53, demonstrated a crucial function in leukemia [16]. According to a previous study, the tubular mitochondrial subpopulation (type II) decreased twice in cells lacking the CTMP protein compared to control cells. A thorough confocal analysis of cellular metabolism revealed the buildup of a heterogeneous network of linked swollen and thick mitochondria, despite the fact that the majority of CTMP-negative cells had filamentous mitochondria [17].

CTMP is an oncogenic driver that stimulates Akt phosphorylation. Akt signaling has been linked to the advancement of oncogenesis because of its ability to control cellular proliferation and prevent apoptosis. In addition to directly phosphorylating and interacting with important components of mitochondria-mediated apoptosis signaling, Akt controls apoptosis. Further research has revealed that Akt signaling contributes to the survival and growth of certain tumor cell types [17]. THEM4 is involved in both apoptosis and Akt1 regulation. Part of the N-terminal domain consists of a flexible and asymmetric secondary structure similar to a domain that binds proteins. With our demonstration of direct THEM4-Akt1 binding via immunoprecipitation and suppression of Akt1 kinase activity, we offer independent proof that THEM4 functions as an Akt1 negative regulator [18].

H. indicus has several medical, biological, and phytopharmaceutical uses; however, all its components have historically been regarded as crude drugs. A wide range of studies on *H. indicus* have exploited a variety of research studies to produce pharmaceuticals [19]. The phylogenetic taxonomy of *H. indicus* revealed that it belongs to the Apocynaceae family, which consists of two varieties: white and black. Sariva is the name given to the white species and Krishna Sariva to the black species [20].

H. indicus contains various phytochemicals in various sections. However, the roots were found to be a major source of phytochemicals. The root section of *H. indicus* contains hexatriacontane acid, lupeol 1-octacosanol, terpenoids, flavonoids, saponin, hemidesmol, tannin and resin, lupeol acetate, and B-amyrin acetate [21].

Anticancer properties were observed in *H. indicus*. The acute myeloid leukemia cell line demonstrated cytotoxic effects as a result of the chemopreventive activity of *H. indicus* extract, according to previous reports [9].

In a study conducted in vivo on rats, anticancer efficacy was demonstrated. Lipid peroxidation, hydroxyl, and superoxide radicals were prevented in a rat model using the methanol extract of the root bark of *H. indicus*. Additionally, in the liver, erythrocytes, and plasma, the extract protects against oxidative stress caused by free radicals [22]. In a further investigation, considerable inhibition of ROS and proinflammatory cytokines in carcinogenesis was observed in polymorphonuclear leukocytes and monocytes treated with Propionibacterium acnes in the presence of *H. indicus* [23].

The post-pharmacological activities showed active protein-ligand complexes that were extracted from the 45 phytoconstituents. In silico analysis revealed the complexes by docking into the target protein, a carboxyl-terminal modulator protein. Quercetin exhibited the highest binding affinities.

CONCLUSION

Acute Myeloid Leukemia (AML) is defined as an elevated count of immature cells (blasts) in the bloodstream and/or the bone marrow. It encompasses a range of conditions with diverse underlying causes and developmental pathways, potentially manifesting at various points in the blood-cell formation process. Genomic investigations of AML have revealed frequent mutations in numerous genes, leading to the identification of novel genomic subtypes, predictive markers, and targets for therapeutic interventions.

In terms of prospects, significant progress has been made in comprehending the genomic characteristics of AML, and the therapeutic potential of quercetin could significantly impact disease prognosis by targeting recurrent alterations. Further exploration of this complexity using MD simulations is warranted.

Acknowledgment

I thank BioNome, Bengaluru, India's Department of Bioinformatics for offering computing resources and assistance with empirical research. I appreciate Miss Samiksha Bhor's help with this project.

Conflict of Interest

The authors declare no conflict of interest

Conflict of Interest

Declared None

Funding

Not applicable

Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
AMY	Acute Myeloid Leukemia
Apaf-1	Apoptotic protease activating factor-1.
BBB	Blood-Brain Barrier
CTMP	Carboxyl-terminal modulator protein
FDA	Food and Drug Administration
H. indicus	*Hemidesmus indicus*
HBA	Hydrogen Bond Acceptors
HBD	Hydrogen Bond Donors
HIA	Human Intestinal Absorption
IMPPAT 2.0	Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0
LOG Kp	Skin permeation
MD Stimulation	Molecular Dynamics Stimulation.
MW	Molecular Weight
PDB	Protein Data Bank
P-GP	Permeability Glycoprotein
PI3K/Akt	Phosphatidylinositol 3-kinase
THEM4	Thioesterase superfamily member 4

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