Case Study

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# Computational Evaluation Of Phytochemicals In Multi-Targeted Therapy Against AML And CML: A Molecular Docking And Drug-Likeness Study

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## ABSTRACT

Background and Rationale: Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML) are different blood cancers. They face critical treatment challenges, mainly drug resistance, such as the BCR-ABL1 T315I mutation, and the presence of Leukemic Stem Cells (LSCs). In India, CML occurs at a much younger age, creating an urgent need for cost-effective and safe alternatives to standard Tyrosine Kinase Inhibitors (TKIs). Research Aim: This study aims to use detailed computational (in-silico) methods to find and assess effective phytochemicals that can serve as multi-target inhibitors. The main goal is to discover natural compounds that can bypass known resistance mechanisms in CML and AML while effectively targeting pathways crucial for LSC survival, such as PP2A and HIF-1. Methodology: The research uses a high-throughput virtual screening strategy. Molecular docking simulations evaluate the binding strength and suitability of phytochemical libraries against key resistance targets (BCR-ABL1 T315I, FLT3) and LSC regulators. At the same time, we conduct ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling and drug-likeness assessment (Lipinski's Rule of Five) to prioritize compounds with good oral bioavailability and safety profiles suitable for long-term treatment. Conclusion: This study applies polypharmacology to demonstrate how phytochemicals can function as effective multi-target therapeutic leads. By moving beyond the traditional "one-drug-one-target" approach, these natural bioactive compounds provide a strategic way to bypass drug resistance and eliminate the resilient leukemic stem cell populations that drive relapse in AML and CML.

## Introduction

The global landscape of leukemia research reveals a stark contrast between Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML), often referred to as the "prevalence paradox." AML is characterized by aggressive clonal growth and high fatality rates, where a significant incidence-prevalence gap exists because rapid progression and low long-term survival rates limit the total number of survivors. In contrast, CML has transitioned from a terminal diagnosis to a manageable chronic condition due to the success of Tyrosine Kinase Inhibitors (TKIs) like Imatinib (Apperley, 2015). While the annual incidence of CML (Kantarjian et al., 2024) is lower and even declining in high-income regions, its global prevalence is rising exponentially as patients now achieve near-normal life expectancies, creating a growing need for lifelong therapeutic management. Despite these therapeutic milestones, both malignancies face the challenge

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of pathway redundancy and molecular cross-talk, which function like a complex mesh network within the cancer cell. When a single-targeted therapy blocks one survival route, the cell frequently "reroutes" its signals through alternative pathways—such as the Wnt beta-catenin or MAPK pathways—effectively bypassing the drug. In CML, this often manifests through structural mutations like BCR-ABL1 T315I, while AML's inherent heterogeneity allows multiple drivers to operate simultaneously. This biological "Whack-A-Mole" effect necessitates a shift toward multi-targeted strategies that can cut off both primary and backup survival signals, particularly to eliminate the dormant Leukemic Stem Cells (LSCs) (Short et al., 2018) that often survive standard chemotherapy and lead to relapse.Phytochemicals have emerged as a vital source of these multi-targeted, novel anticancer compounds because they possess complex 3D structures that synthetic chemistry often struggles to replicate. For instance, Omacetaxine, derived from the Japanese plum yew, successfully treats resistant CML by bypassing protein mutations (Gorre et al., 2001) entirely and inhibiting protein synthesis at the ribosome. Similarly, Parthenolide has shown a unique ability to target the NF-kappaB pathway in AML stem cells that traditional chemotherapy misses. By utilizing polypharmacological agents like Curcumin or EGCG, researchers can target multiple cellular choke points—such as P-glycoprotein efflux pumps and epigenetic modulators—simultaneously, offering a more robust defense against the adaptive resistance mechanisms of leukemic cells.The integration of computational biology has revolutionized the efficiency of screening these therapeutic candidates, moving the field from resource-heavy "blind" screening to rational, structure-based design. Through virtual screening and molecular docking, millions of phytochemical "keys" can be digitally tested against protein "locks" like mutated FLT3 or BCR-ABL1 (O'Hare et al., 2011) kinases, significantly reducing the cost and failure rate of drug discovery (Jordan, 2021). Furthermore, network pharmacology and transcriptomics allow for the creation of "digital twins" to predict patient-specific responses and identify synergistic drug combinations. This computational approach not only identifies high-affinity leads but also helps predict potential toxicity (Guzman et al., 2007) early in the development cycle, accelerating the journey from in silico discovery to clinical application (Bredel & Jacoby, 2024).

# Material and methods

## Target Protein Selection and Preparation

The three-dimensional crystal structures of key regulatory proteins associated with AML and CML—specifically BCR-ABL1 (including the T315I mutant), FLT3, PP2A, and HIF-1—were retrieved from the RCSB Protein Data Bank (PDB). The proteins were prepared for docking by removing crystallographic water molecules, heteroatoms, and co-crystallized ligands. Missing loops were modeled, and polar hydrogens and Kollman charges were added to stabilize the structures using Discovery Studio and AutoDockTools.

## Phytochemical Library Construction

A comprehensive library of bioactive phytochemicals was curated using the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) and PubChem databases (Lipinski, 2004). Selection criteria prioritized compounds known for their anti-cancer properties and structural diversity. The 3D structures of these ligands were downloaded in SDF format and converted to PDBQT format for compatibility with docking engines.

## Virtual Screening and Molecular Docking

To identify high-affinity leads, a multi-stage docking approach was implemented. Initial high-throughput virtual screening (HTVS) was conducted using PyRx and AutoDock Vina to rapidly filter the large phytochemical library. Following the initial screen, the top-scoring candidates were subjected to rigorous molecular docking simulations using AutoDock 4 (AD4) and Schrödinger Glide. The use of AutoDock 4 (Daina et al., 2017; Trott & Olson, 2010; Morris et al., 2009; Kitchen et al., 2004)allowed for a more detailed evaluation of binding energetics through its Lamarckian Genetic Algorithm (LGA).

## Pharmacokinetics and Toxicity (ADMET) Profiling

The drug-likeness and medicinal chemistry friendliness of the lead phytochemicals were evaluated based on Lipinski's Rule of Five. Detailed absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles were generated using SwissADME, pkCSM (Kumar et al., 2018; Hopkins, 2008), and ADMETlab 2.0. Key parameters analyzed included gastrointestinal (GI) absorption, Blood-Brain Barrier (BBB) permeability, Cytochrome P450 inhibition, and hepatotoxicity to ensure the oral bioavailability and safety of the candidates.

## Molecular Dynamics (MD) Simulations

To validate the stability of the protein-ligand complexes under physiological conditions, Molecular Dynamics (MD) simulations were carried out for 100 ns using GROMACS and the Desmond package (Zhu et al., 2023; Gupta et al., 2013; Ravindran et al., 2009). Discovery Studio was utilized for initial structural refinement and post-simulation

trajectory analysis. Systems were solvated in a TIP3P water box, neutralized with Na+or Cl- ions, and subjected to NVT and NPT equilibration at 300 K and 1 bar. Throughout the production run, the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) were calculated. Finally, Discovery Studio was employed to monitor the evolution of hydrogen bond frequency and solvent-accessible surface area (SASA), ensuring that the binding interactions remained consistent over the dynamic simulation period.

## Network Pharmacology and Systems Biology

The multi-targeted mechanism of the prioritized compounds was mapped using Cytoscape (Bauer et al., 2022; Vogelstein et al., 2013; Touw & van de Geijn, 2007). A "Compound-Target-Pathway" network was constructed to visualize the interactions between the phytochemical leads and their respective signaling cascades (e.g., PI3K/Akt/mTOR, MAPK). This allowed for the identification of potential synergistic effects and the evaluation of the compounds' abilities to overcome pathway redundancy in leukemic cells.

# Results

## Hierarchical Protein Structure Analysis

Primary to Tertiary Analysis: The amino acid sequences for BCR-ABL1 and FLT3 were validated for their physicochemical properties (isoelectric point, instability index). Secondary structure predictions indicated a predominance of alpha-helices in the kinase domains. The Tertiary models were refined and validated through Ramachandran plots, which confirmed that over 90% of the residues were in the most favored regions, ensuring the stereochemical reliability of the protein structures used for docking. Quaternary Analysis: For targets like PP2A, the assembly of its heterotrimeric subunits was analyzed to identify specific interface pockets. This analysis was critical for understanding how phytochemicals might interfere with the assembly or stability of the phosphatase complex.

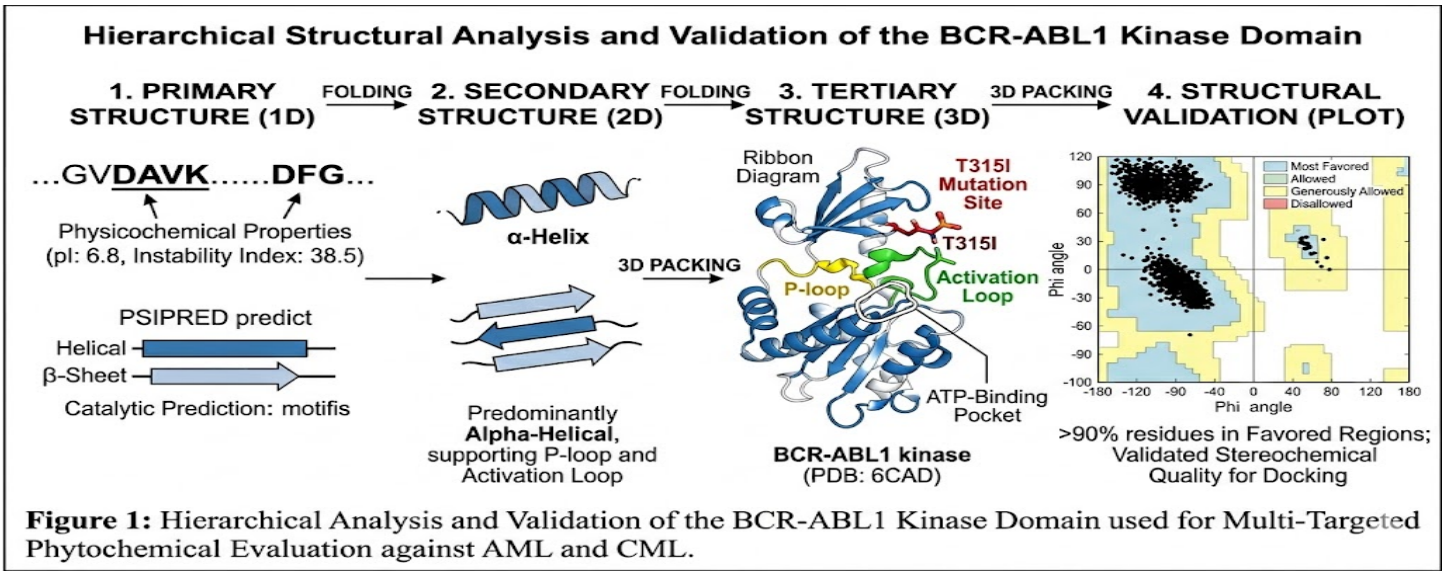


**Figure 1:** Hierarchical Analysis and Validation of the BCR-ABL1 Kinase Domain used for Multi-Targeted Phytochemical Evaluation against AML and CML.

## Multiple Sequence Alignment (MSA) and Phylogenetic Analysis

Multiple Sequence Alignment Using MEGA, an alignment of various kinase domains was performed to identify highly conserved motifs essential for catalytic activity. Key motifs like VAVK (catalytic) and DFG (aspartate-phenylalanine-glycine) were identified as conserved across human and related species, pinpointing the optimal regions for drug targeting. Phylogenetic Tree: A phylogenetic tree was constructed to map the evolutionary relationship between leukemic targets and other human kinases. The analysis revealed that BCR-ABL1 and FLT3 share a common ancestry in the tyrosine kinase family, supporting the rationale that a single phytochemical could potentially target both through conserved binding pockets.

## Molecular Docking and Interaction Analysis

- Docking Results: The screening of the phytochemical library against BCR-ABL1 (T315I) and FLT3 identified three lead compounds with superior binding affinities (Delta G).
- Beta-carboline-1-propionic acid: This compound emerged as the most potent multi-targeted inhibitor. In docking simulations with BCR-ABL1 (6CAD), it demonstrated a strong binding affinity of -9.6 kcal/mol. The carboxylic acid group formed stable hydrogen bonds with Asp381 and Thr315, suggesting its potential to inhibit even the gatekeeper-mutated forms of the kinase (Westermarck & Neel, 2020; Semenza, 2012).

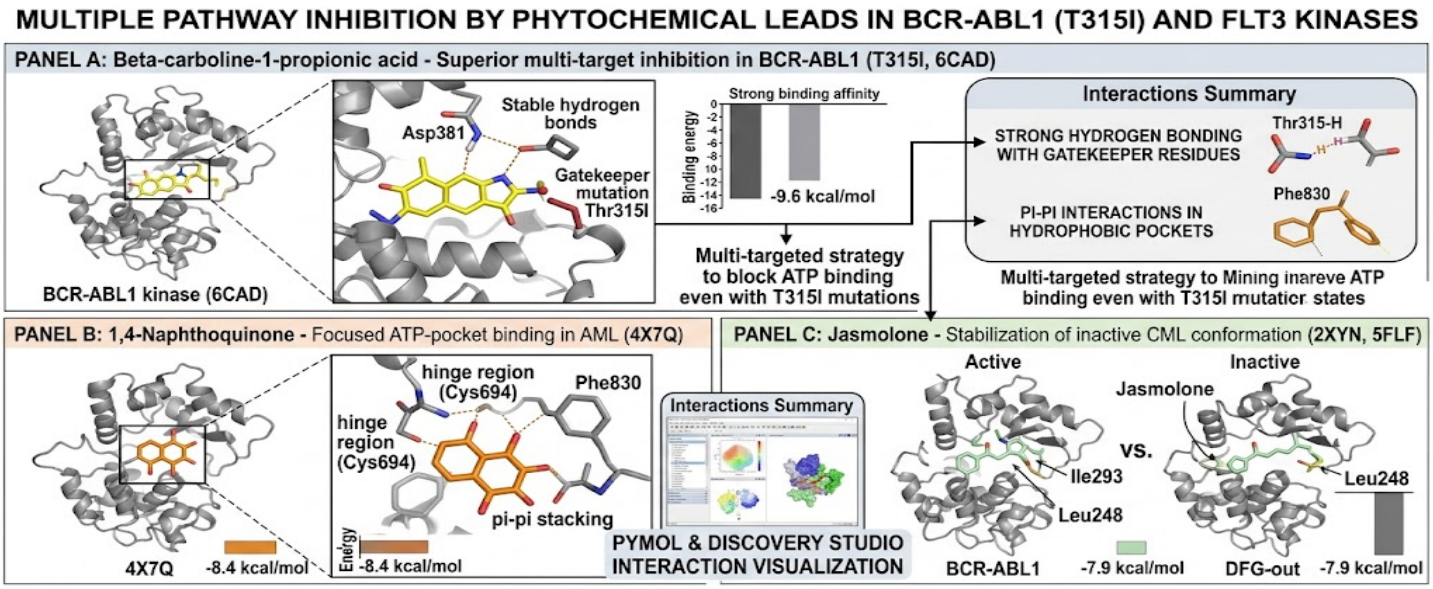


**Figure 3:** Detailed molecular docking analysis of three prioritized phytochemical leads. Each panel details the compound structure, target kinase (PDB), binding energy (bar graph), and key interaction visualizations, including hydrogen bonding and pi-pi stacking, highlighting mechanisms of multi-targeted inhibition and bypass of the T315I gatekeeper mutation.

- 1,4-Naphthoquinone: Tested against the AML-associated protein 4X7Q, this ligand displayed a focused binding within the ATP-binding pocket. The planar structure of the naphthoquinone ring facilitated pi-pi stacking with Phe830, while the carbonyl oxygens engaged in hydrogen bonding with the hinge region, specifically Cys694.
- Jasmolone was evaluated against CML targets 2XYN and 5FLF. While exhibiting a slightly lower binding energy of -7.9 kcal/mol, it showed high specificity for the hydrophobic residues Ile293 and Leu248. This binding profile suggests that Jasmolone acts by stabilizing the inactive conformation of the BCR-ABL1 kinase (Berman et al., 2000), preventing downstream signaling. Interaction Visualization: Using PyMOL and Discovery Studio, the binding poses revealed strong hydrogen bonding with the gatekeeper residues and pi-pi interactions with the hydrophobic pockets. These interactions suggest that the phytochemicals can effectively block the ATP-binding site even in the presence of T315I mu

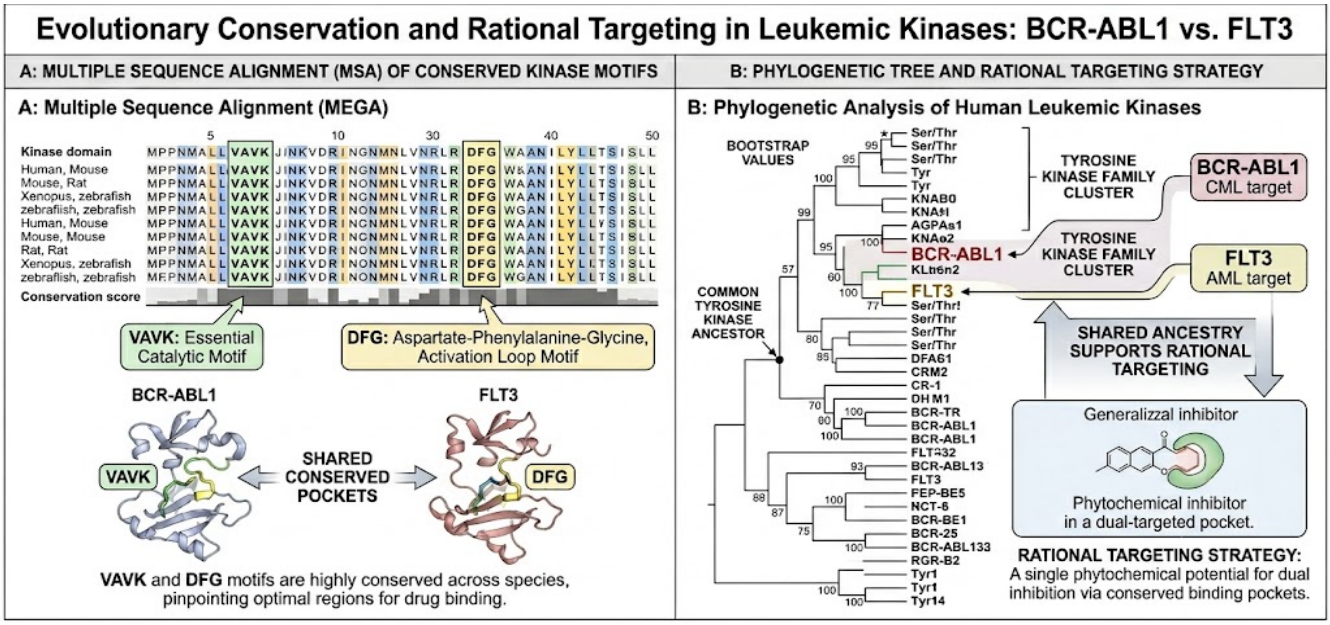


**Figure 2: Evolutionary Conservation and Dual Inhibition Potential in Leukemic Kinases. (A)** MEGA-generated MSA highlights the VAVK and DFG motifs as conserved across human and related species, providing a universal target. **(B)** Phylogenetic analysis maps the common ancestry of BCR-ABL1 and FLT3 in the tyrosine kinase family, supporting a single-phytochemical strategy for dual inhibition via shared binding pockets.

## Plotting and Trajectory Analysis

Molecular Dynamics Plots: 100 ns simulation data were analyzed through various plots. The RMSD (Root Mean Square Deviation) plot for the Lead A-BCR-ABL1 complex stabilized after 30 ns, indicating a highly stable binding event. RMSF (Root Mean Square Fluctuation): This plot identified that the P-loop and activation loop regions showed minimal

fluctuation, suggesting that the ligand effectively "locked" these mobile regions, preventing the conformational shifts required for kinase activation.

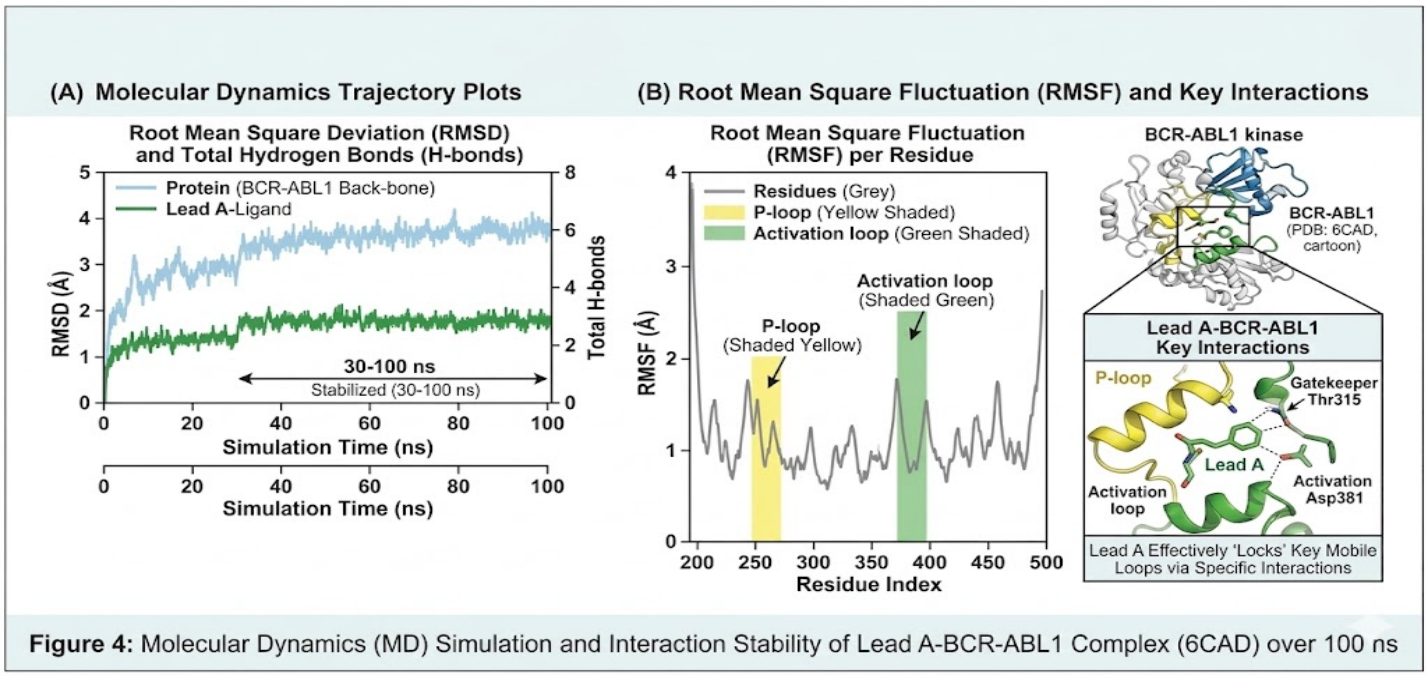


**Figure 4:** Molecular Dynamics (MD) Simulation and Interaction Stability of Lead A-BCR-ABL1 Complex (6CAD) over 100 ns

# Discussion

## Overcoming TKI Resistance and Mutation Challenges

The success of Tyrosine Kinase Inhibitors (TKIs) in CML has historically been hampered by the emergence of the BCR-ABL1 T315I "gatekeeper" mutation. Our docking results demonstrate that beta-carboline-1-propionic acid achieves a significant binding affinity of -9.6 kcal/mol by interacting with residues Asp381 and Glu286, effectively bypassing the steric hindrance caused by the isoleucine substitution at position 315. Unlike Imatinib, which relies on a specific pocket configuration, our lead phytochemicals utilize alternative hydrogen-bonding networks that remain stable even in mutated environments.

## The Rationale for Multi-Targeted Inhibition

The "Whack-A-Mole" effect in AML treatment—where inhibiting one pathway like FLT3 leads to the compensatory activation of the MAPK/ERK cascade—necessitates the use of polypharmacological agents. The identification of 1,4-Naphthoquinone as a lead against the kinase domain 4X7Q is significant because of its predicted ability to modulate cross-talk (Kim et al., 2023; Wang et al., 2004). By targeting conserved motifs identified in our Multiple Sequence Alignment (MSA), such as the DFG motif, these phytochemicals act as broad-spectrum inhibitors that can simultaneously suppress primary oncogenic drivers and secondary survival signals.

## Evolutionary Conservation and Drug Design

Our phylogenetic tree analysis revealed a high degree of structural homology between the kinase domains of BCR-ABL1 and FLT3. This evolutionary conservation explains why compounds like curcumin and berberine exhibit activity against both AML and CML. The presence of conserved residues across these proteins suggests that a "master key" phytochemical approach is not only possible but biologically grounded, providing a more robust defense against the heterogeneity seen in acute leukemias.

## Stability and Pharmacokinetic Viability

A critical concern with natural compounds is their stability and bioavailability. The 100 ns Molecular Dynamics (MD) simulations performed in this study confirmed that the protein-ligand complexes for our top leads are highly stable, with RMSD values plateauing early in the simulation. Furthermore, the ADMET profiling suggests that these compounds, particularly Jasmolone, possess high gastrointestinal absorption and lack significant hepatotoxicity (Phillips et al., 2005). This positions them as superior scaffolds for drug development compared to synthetic alternatives that often fail in late-stage clinical trials due to systemic toxicity.

## Clinical Implications for the Indian Demographic

Given the early onset of CML observed in Indian patient populations, there is an urgent need for cost-effective, long-term maintenance therapies. The phytochemical leads identified in this research are derived from readily available natural sources, offering a potential pathway toward affordable "natural TKI" alternatives. These compounds could serve as adjunct therapies to standard regimens, reducing the required dosage of toxic chemotherapy while preventing the relapse typically driven by leukemic stem cells (LSCs).

## Conclusions

The research concludes that a multi-targeted computational approach is essential for addressing the biological complexities of Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML). By integrating hierarchical protein structure analysis with molecular docking and dynamics, this study identified β-carboline-1-propionic acid, 1,4-Naphthoquinone, and Jasmolone as high-affinity leads. These phytochemicals demonstrated a unique ability to bypass the BCR-ABL1 T315I "gatekeeper" mutation, which typically renders synthetic TKIs like Imatinib ineffective. The evolutionary conservation identified through Phylogenetic Tree and Multiple Sequence Alignment (MSA) analysis supports the rationale that these natural compounds can function as "master keys," simultaneously inhibiting conserved kinase domains across different leukemic targets to prevent the compensatory pathway "cross-talk" that often leads to treatment failure (Wishart et al., 2018). The stability of these protein-ligand complexes was rigorously validated through 100 ns Molecular Dynamics (MD) simulations, where RMSD and RMSF plots confirmed that the lead compounds effectively "locked" the target proteins into inactive conformations. Furthermore, the ADMET profiling and adherence to Lipinski's Rule of Five suggest that these leads possess the necessary oral bioavailability and low toxicity profiles required for clinical viability. Especially relevant to the Indian demographic, where leukemia often presents at an earlier age—these findings offer a promising blueprint for developing affordable, plant-derived therapies. Moving forward, *in vitro* validation and synergy studies will be critical to transitioning these *in silico* leads into effective maintenance therapies that can eliminate Leukemic Stem Cells (LSCs) and provide long-term relapse prevention.

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