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In-Silico Evaluation of Anti-Leukemic Potential of Selected Phytoconstituents from Withania Somnifera (L.)

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ABSTRACT:

Introduction: Leukemia, a diverse group of hematological malignancies, poses a persistent clinical challenge despite advances in therapy. The exploration of natural products as anti-cancer agents has gained momentum, with Withania somnifera (L.) (Ashwagandha), a key Rasayana herb in Ayurveda, showing potential due to its rich phytochemical profile. Bioactive compounds from this plant exhibit antioxidant, immunomodulatory and cytotoxic properties, warranting investigation for anti-leukemic activity.

Methods: Eleven major phytoconstituents of Withania somnifera (L.) were evaluated against the therapeutic target Leukotriene A4 Hydrolase (LTA4H; PDB ID: 6TJU). Three-dimensional structures were retrieved from PubChem in SDF format only and prepared for docking. Molecular docking was performed using PyRx 0.8 (with the in-built AutoDock Vina engine) to assess binding energy, hydrogen bonding and RMSD. Pharmacokinetic profiling was performed using SwissADME to evaluate molecular weight, lipophilicity (iLogP), hydrogen-bond donors/acceptors and compliance with Lipinski's Rule of Five. Probable molecular targets were predicted using SwissTargetPrediction.

Results: Withanolide L demonstrated the strongest binding affinity (-9.8 kcal/mol) to LTA4H (6TJU), followed by Somniferanolide (-9.5 kcal/mol), Sominone (-9.4 kcal/mol), and Withanolide Q & R (-9.3 kcal/mol). All eleven compounds showed binding energies between -8.5 and -9.8 kcal/mol. Most compounds adhered to Lipinski's criteria with favourable ADME profiles. These results indicate the drug-likeness of these phytoconstituents and their ability to interact effectively with the active site of Leukotriene A4 Hydrolase (LTA4H).

Conclusion: The study highlights Withanolide L, Somniferanolide and Sominone as the most promising lead compounds for anti-leukemic drug development. Their strong binding affinities and favourable pharmacokinetic properties support further in vitro experimental validation. This study reinforces the therapeutic potential of Withania somnifera (L.) in hematological malignancies through computational drug discovery.

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1. INTRODUCTION

Leukemia comprises a group of hematological malignancies originating in the bone marrow and characterized by uncontrolled proliferation of immature or abnormal white blood cells. These cells interfere with the normal production and function of blood components, leading to clinical manifestations such as anemia, recurrent infections and bleeding tendencies. Leukemia is broadly categorized into acute and chronic types, based on disease progression, and into lymphoid or myeloid types, based on the cell lineage affected. Despite significant advancements in chemotherapy, immunotherapy and stem cell transplantation, many forms of leukemia still carry a poor prognosis, particularly due to drug resistance, relapse and adverse treatment effects [1,2]. As a result, the search for novel, less toxic and more effective therapies remains a pressing priority in hematological oncology [3,4].

Natural products have long been a vital source for drug discovery, especially in oncology. More than 60% of current anticancer agents are derived from natural sources or their structural analogs [3]. Plants, in particular, produce a wide range of secondary metabolites with diverse pharmacological activities, including anticancer, anti-inflammatory, antioxidant and immunomodulatory effects [5,6]. These compounds serve as lead molecules for developing targeted therapies and often exhibit multitargeted mechanisms of action. The traditional Indian system of medicine, Ayurveda, offers a rich repository of medicinal plants, many of which have demonstrated cytotoxic and chemopreventive properties in modern pharmacological studies [7,8].

Withania somnifera (L.), commonly known as Ashwagandha or Indian ginseng, is a prominent Rasayana herb in Ayurveda, traditionally used to promote longevity, vitality and resistance to stress [7,9]. Belonging to the family Solanaceae, Ashwagandha has been widely studied for its adaptogenic, neuroprotective, anti-inflammatory and anticancer properties [7,9,10]. Several preclinical studies have reported that extracts and bioactive compounds from *Withania somnifera* (L.) can inhibit cancer cell proliferation, induce apoptosis, arrest cell cycle progression and suppress angiogenesis in a variety of cancer types, including breast, prostate, lung, colon and hematological cancers such as leukemia [3,6,8,10].

The anticancer potential of *Withania somnifera* (L.) is attributed to its diverse array of phytochemicals, including withanolides (such as withaferin A), alkaloids, flavonoids, phenolic acids and steroidal lactones [5,6]. These compounds modulate key cellular signalling pathways including NF-κB, PI3K/Akt, p53 and caspase cascades, which are commonly dysregulated in cancer cells [10,11,12]. In the context of leukemia, certain constituents of Ashwagandha have shown promising results *in-vitro*, exhibiting the ability to induce differentiation in leukemic blasts, inhibit telomerase activity and sensitize leukemic cells to chemotherapeutic agents [1,13,14]. However, many of these studies have been limited to cell-based models and further exploration of specific molecular targets through computational approaches is warranted [4,15].

A critical mediator of inflammation and leukocyte signalling implicated in cancer progression is Leukotriene A4 Hydrolase (LTA4H)- a zinc-dependent enzyme that catalyzes the conversion of leukotriene A4 (LTA4) to leukotriene B4 (LTB4), a potent pro-inflammatory lipid mediator. LTB4 promotes chemotaxis of leukocytes, enhances vascular permeability and stimulates cytokine production. Recent evidence suggests that chronic inflammation, mediated in part by LTA4H activity, plays a key role in the pathogenesis of hematological malignancies, including leukemia [2,12]. Elevated levels of LTB4 have been associated with increased proliferation and survival of leukemic cells. Additionally, LTA4H has been found to be overexpressed in various cancers and is considered a potential therapeutic target in inflammation-driven carcinogenesis [2,16].

Targeting LTA4H may provide dual benefits: suppression of inflammation and inhibition of cancer progression. While some synthetic inhibitors of LTA4H have been developed, their side effects and limited efficacy have prompted researchers to explore natural inhibitors [2]. This makes *Withania somnifera* (L.), with its anti-inflammatory and anticancer phytoconstituents, a logical candidate for investigation as a source of natural LTA4H inhibitors [8,9].

In recent years, *in-silico* approaches have gained popularity in early-stage drug discovery due to their cost-effectiveness and rapid turnaround [15,17]. Molecular docking allows the prediction of binding affinity and interaction profiles between ligands (such as phytochemicals) and target proteins (such as LTA4H) [18,19]. It helps in identifying promising lead compounds that may interact favourably with active sites of key disease-related proteins. Furthermore, computational ADME (Absorption, Distribution, Metabolism and Excretion) analysis and drug-likeness evaluation based on Lipinski's Rule of Five and other pharmacokinetic parameters help in determining the potential of compounds to become orally active drugs [20,21].

The present study was designed to assess the anti-leukemic potential of selected bioactive compounds from *Withania somnifera* (L.), using molecular docking against Leukotriene A4 Hydrolase (PDB ID: 6TJU) [2,4]. This target was chosen due to its

established role in pro-inflammatory signalling, leukocyte recruitment and tumour progression. Eleven major phytoconstituents were selected based on literature evidence and their molecular interactions with LTA4H were analyzed using docking software. Key parameters such as binding affinity (kcal/mol), hydrogen bonding interactions and root-mean-square deviation (RMSD) were evaluated [18,19]. Additionally, pharmacokinetic profiling was carried out using SwissADME [21] to determine drug-likeness, lipophilicity, molecular weight and hydrogen-bonding characteristics. Probable molecular targets were predicted using SwissTargetPrediction [22].

The use of *in-silico* methods to screen *Withania somnifera* (L.), compounds against LTA4H provides valuable insight into their potential mechanism of action in leukemia therapy. If successful, this approach may yield promising natural inhibitors of leukotriene-mediated inflammation and offer novel leads for anti-leukemic drug development with fewer side effects [4,14,23].

Aim and Objectives

The aim of the present study is to evaluate selected bioactive compounds from *Withania somnifera* (L.), for their potential to inhibit Leukotriene A4 Hydrolase (LTA4H), a key enzyme involved in inflammation-associated leukemogenesis, using computational drug discovery approaches.

The specific objectives are as follows:

- To retrieve and prepare the crystal structure of Leukotriene A4 Hydrolase (PDB ID: 6TJU) for molecular docking analysis.
- To screen eleven major phytochemicals from *Withania somnifera* (L.), against LTA4H using molecular docking to determine binding affinity and key interactions.
- To analyze docking scores, hydrogen-bond formation and RMSD values to assess ligand–protein stability.
- To evaluate the pharmacokinetic properties of these compounds, including ADME profiling and drug-likeness according to Lipinski's Rule.
- To identify the most promising phytoconstituents with potential anti-leukemic activity for further *in vitro* experimental validation.

2. MATERIALS AND METHODS

The present study was designed to assess the anti-leukemic potential of selected phytochemicals from *Withania somnifera* (L.), by targeting Leukotriene A4 Hydrolase (LTA4H) using *in-silico* tools. The methodology involved selection of phytoconstituents, ligand preparation, protein retrieval and optimization, molecular docking, ADME analysis and target prediction. All steps were performed using freely available, validated bioinformatics tools, namely Discovery Studio Visualizer (for protein preparation) PyRx 0.8 (molecular docking) [18], SwissADME (pharmacokinetic profiling) [21] and SwissTargetPrediction (target prediction) [22].

2.1 Selection and Retrieval of Phytochemicals

Phytoconstituents of *Withania somnifera* (L.), were selected based on a literature review and curated databases [5,6,8]. The Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database (https://cb.imsc.res.in/imppat/) was utilized to retrieve bioactive constituents reported in *Withania somnifera* (L.). IMPPAT is a curated resource that provides high-quality information on Indian medicinal plants, their phytochemicals and therapeutic uses.

The 2D and 3D structures of these compounds were retrieved in Structure-Data File (SDF) format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SDF format was selected because it carries 3-D coordinates suitable for direct use in PyRx and is fully compatible with the Open Babel converter integrated within PyRx. The SDF files were then imported into PyRx and converted into PDBQT format for molecular docking using the integrated Open Babel module [18].

2.2 Ligand Optimization and Preparation

After downloading the structures, energy minimization of each ligand was carried out within PyRx 0.8 using its built-in Open Babel module (Universal Force Field, UFF). The minimized SDF ligands were then loaded into PyRx's "Open Babel" panel

and converted directly to AutoDock-compatible PDBQT format. PyRx automatically assigned Gasteiger partial charges, detected rotatable bonds and set the appropriate torsional flexibility during this conversion, eliminating the need for any external preparation through standalone AutoDock Tools [18,19].

The main aim of ligand preparation was to ensure that each phytochemical existed in a stable conformation with minimum energy and correct protonation states at physiological pH (~7.4). Hydrogen atoms were added wherever necessary and unnecessary water molecules or salts were removed.

2.3 Target Protein Retrieval and Preparation

The crystal structure of the target protein Leukotriene A4 Hydrolase (LTA4H) was retrieved from the Protein Data Bank (PDB ID: 6TJU) in .pdb format. This structure was selected because of its high resolution and relevance in inflammation-mediated leukemogenesis.

Protein preparation was performed entirely within PyRx 0.8.

- The downloaded PDB file was imported through the "Load Molecule" option;
- Co-crystallized water molecules and hetero-atoms were removed;
- Polar hydrogens were added
- Partial charges were assigned automatically by PyRx's AutoDock pre-processing module.
- The cleaned protein was then saved as a macromolecule in PDBQT format. No external AutoDock Tools / MGLTools step was required [18].

The active site of LTA4H was identified based on the position of the co-crystallized ligand and on the known catalytic domain as reported in PDBsum and related literature. The coordinates for the grid box were adjusted to cover the catalytic pocket for unbiased screening of all ligands.

2.4 Molecular Docking Using PyRx

Molecular docking was carried out using PyRx 0.8 (The Scripps Research Institute) [18], which uses the AutoDock Vina engine internally [19]. PyRx provides an integrated graphical interface for ligand import, energy minimization, PDBQT conversion, grid-box configuration, virtual screening and pose visualization within a single environment.

Docking parameters:

- Grid box center coordinates: adjusted to encompass the known active site of LTA4H (6TJU).
- Grid dimensions: chosen to allow complete entry of the ligand into the active site.
- Exhaustiveness: set to the PyRx default value of 8, which balances computational speed and search accuracy.
- Number of binding modes: 9 poses per ligand were generated (PyRx/AutoDock Vina default).

Each ligand was docked individually against the prepared protein and the best pose was selected based on the lowest (most negative) binding energy in kcal/mol [19,24]. RMSD (upper/lower bound) values produced by Vina were also recorded to assess pose stability. Ligand–protein interactions, including hydrogen bonds, hydrophobic contacts and π–π stacking, were inspected directly within the PyRx interface ("View Pose / Analyse") and Discovery Studio Visualizer.

2.5 ADME and Drug-Likeness Prediction

To evaluate the pharmacokinetic profile and drug-likeness of the selected phytochemicals, SwissADME (http://www.swissadme.ch/) was used [21]. This freely accessible web tool evaluates the following parameters:

- Lipophilicity (iLogP)
- Molecular weight (MW)
- Hydrogen-bond donors (HBD)

- Hydrogen-bond acceptors (HBA)
- Lipinski's Rule of Five compliance

Canonical SMILES strings of each phytoconstituent were directly copied from PubChem and pasted into the SwissADME input window. The results were tabulated to identify compounds meeting the standard drug-likeness criteria. Special attention was paid to the number of Lipinski violations, since compounds with zero violations are more likely to be orally bioavailable [20,25].

Additionally, the BOILED-Egg model within SwissADME was used to predict passive gastrointestinal absorption and blood-brain barrier (BBB) permeability, which are critical for systemic drug delivery [21].

2.6 Target Prediction Analysis

To further validate the relevance of LTA4H as a predicted target, each ligand's possible molecular targets were predicted using SwissTargetPrediction (http://www.swisstargetprediction.ch/) [22]. This tool uses a combination of 2-D and 3-D similarity measures against known bioactive molecules to predict probable macromolecular targets in the human proteome.

The canonical SMILES of each phytochemical was submitted and the top 15 predicted targets were extracted. These predicted targets were then compared with proteins reported to be involved in leukemia or inflammatory pathways [4,12]. Particular attention was paid to whether LTA4H, related zinc-dependent metallopeptidases or other inflammation-driving enzymes appeared in the predicted target list, since this would further reinforce the docking outcome.

2.7 Data Management and Statistical Analysis

All docking scores (binding affinities), ADME profiles and target predictions were compiled in Microsoft Excel. Descriptive statistics were used to present binding energies, the number of hydrogen bonds, molecular weights and compliance with Lipinski's rules. Because the present work is an exploratory in-silico screen of eleven phytoconstituents against a single target, no inferential statistical tests were applied; the results are reported as direct, per-ligand parameters.

Protein–ligand interaction profiles (hydrogen-bond donors/acceptors, hydrophobic contacts and binding-pocket residues) were extracted directly from the PyRx output and verified using the built-in 3-D viewer.Discovery Studio Visualizer was applied for final selected compound

3. OBSERVATION AND RESULTS

The present *in-silico* investigation evaluated eleven phytoconstituents from *Withania somnifera* (L.), for their anti-leukemic potential by molecular docking against Leukotriene A4 Hydrolase (LTA4H; PDB ID: 6TJU), a protein associated with inflammation-driven carcinogenesis. The analysis covered binding affinity, RMSD values, molecular weight, hydrogen-bonding potential, lipophilicity (iLogP) and drug-likeness based on Lipinski's Rule of Five, along with predicted drug targets.

3.1 Molecular Docking Analysis

The docking scores ranged from -8.5 to -9.8 kcal/mol, indicating high binding affinity of the selected compounds toward 6TJU (LTA4H). Withanolide L showed the highest binding affinity at -9.8 kcal/mol, followed closely by Somniferanolide (-9.5 kcal/mol), Sominone (-9.4 kcal/mol), Withanolide Q and R (-9.3 kcal/mol) and Withanolide J (-9.1 kcal/mol). These values suggest that these molecules interact strongly with the active site of LTA4H.

3.2 RMSD Values

In molecular docking, lower RMSD values indicate a more reliable and reproducible binding pose; values below ~2 Å are considered excellent, 2–3 Å acceptable, and values above ~5 Å reflect poses that should be interpreted with caution [24]. Among the eleven compounds screened, the (2R)-hydroxyethyl derivative (RMSD = 1.808 Å) and Withanolide Q (RMSD = 2.281 Å) showed the most stable docking orientations, while Withanolide L (RMSD = 3.75 Å) demonstrated an acceptable pose despite its highest binding affinity. Compounds with higher RMSD values (Sominone and Viscosalactone B, ~30 Å) likely reflect the flexible ring systems or pendant side-chains of these withanolides shifting the centroid relative to the reference.

3.3 Lipinski's Rule of Five Compliance

All selected compounds complied with Lipinski's Rule of Five, indicating favourable predicted oral bioavailability. Each compound possessed:

- Molecular weight < 500 g/mol
- Hydrogen-bond donors ≤ 5 (one compound, Viscosalactone B, showed 7 donors and was flagged in the discussion)
- Hydrogen-bond acceptors ≤ 10
- iLogP < 5

This supports the hypothesis that these compounds are potentially active.

3.4 Hydrogen Bonding and Drug-Likeness

Most of the compounds demonstrated 5–6 hydrogen-bond donors and 2–3 hydrogen-bond acceptors, enabling formation of multiple stabilising interactions with amino-acid residues in the active site of LTA4H (6TJU). Moderate iLogP values (3.21–3.69) suggest a balanced lipophilic/hydrophilic profile consistent with good passive membrane permeability.

3.5 Predicted Drug Targets

The Swiss Target Prediction analysis revealed that many of the compounds have predicted affinity for:

- Protein Kinase C alpha (PKCα)- involved in proliferation and apoptosis regulation in cancer cells
- Neprilysin- a zinc-dependent metallopeptidase linked to peptide metabolism and potentially involved in oncogenesis

Such targeting is especially relevant in leukemia, where dysregulation of kinases and inflammation-associated metallopeptidases contributes to disease progression.

3.6 Lead Compounds Identified

Based on the combined evaluation of binding energy, RMSD, hydrogen-bonding potential and drug-likeness, the following compounds emerged as top candidates:

- Withanolide L- highest docking score (-9.8 kcal/mol), acceptable pose
- Somniferanolide - strong binding, high donor count
- Sominone - dual target prediction, excellent docking score
- (2R)-hydroxyethyl derivative - best RMSD
- Withanolide Q and R - balanced ADME and docking

These compounds are considered promising leads for further *in vitro* validation against leukemia via inhibition of the LTA4H protein (6TJU).

Table 1: Final findings from the *in-silico* investigation of *Withania somnifera* (L.)

Sr. No.	Compound Name	Binding Energy (kcal/mol)	RMSD (Å)	MW (g/mol)	H-B Donor	H-B Acceptor	iLogP	Lipinski Violation	Predicted Drug Targets (SwissTargetPrediction)
1	Somniferanolide	-9.5	6.047	468.6	6	2	3.33	0	Protein kinase C alpha; Protein kinase C
2	Somniferawithanolide	-8.5	9.915	470.6	6	3	3.21	0	Protein kinase C alpha; Protein kinase C

Sr. No.	Compound Name	Binding Energy (kcal/mol)	RMSD (Å)	MW (g/mol)	H-B Donor	H-B Acceptor	iLogP	Lipinski Violation	Predicted Drug Targets (SwissTargetPrediction)
3	Withanolide Q	-9.3	2.281	470.6	6	3	3.36	0	Protein kinase C alpha; Protein kinase C
4	Withanolide R	-9.3	8.728	470.6	6	2	3.69	0	Protein kinase C alpha; Protein kinase C
5	Viscosalactone B	-8.7	30.271	488.6	7	3	3.23	0	Neprilysin; Protein kinase C alpha
6	Sominone	16.-9.4	30.281	458.6	5	3	3.59	0	Neprilysin; Protein kinase C alpha
7	Withanolide derivative (compound 7)*	-9.2	9.2	470.6	6	2	3.41	0	Protein kinase C alpha; Protein kinase C
8	Withanolide L	-9.8	3.75	452.6	5	2	3.32	0	Protein kinase C alpha
9	(2R)-hydroxyethyl withanolide derivative**	-9.6	1.808	470.6	6	3	3.33	0	Protein kinase C alpha
10	Withanolide J	-9.1	5.238	470.6	6	3	3.50	0	Protein kinase C alpha
11	Withaferin A	-9.0	8.808	470.6	6	2	3.24	0	Protein kinase C alpha

** Compound 7: (1S,2S,4S,5R,10R,11S,14R,15R,18S)-5-hydroxy-15-[(1S)-1-[5-(hydroxymethyl)-4-methyl-6-oxo-2,3-dihydropyran-2-yl]ethyl]-10,14-dimethyl-3-oxapentacyclo[9.7.0.0², ⁴.0 ⁵,¹ ⁰.0¹ ⁴,¹ ⁸]octadec-7-en-9-one.*

*** Compound 9: (2R)-2-[(1S)-1-[(8R,9S,10R,13S,14R,17S)-14,17-dihydroxy-10,13-dimethyl-1-oxo-4,7,8,9,11,12,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-1-hydroxyethyl]-4,5-dimethyl-2,3-dihydropyran-6-one.*

4. DISCUSSION

In the present study, an *in-silico* docking analysis was conducted to explore the potential of bioactive constituents from *Withania somnifera* (L.), against Leukotriene A4 Hydrolase (LTA4H)-a bifunctional zinc-dependent enzyme with epoxide hydrolase and aminopeptidase activity, implicated in inflammation-driven carcinogenesis [2]. The docking results provide critical insights into the anti-leukemic properties of these phytocompounds.

All eleven compounds exhibited strong binding affinities against LTA4H, ranging from -8.5 to -9.8 kcal/mol, indicating potential inhibition of the enzyme's active site. The top binders were Withanolide L (-9.8 kcal/mol), Somniferanolide (-9.5 kcal/mol), Sominone (-9.4 kcal/mol) and Withanolide Q and R (-9.3 kcal/mol). Binding energies more negative than -9.0 kcal/mol are generally considered promising for lead identification in virtual screening campaigns [15,19].

Hydrogen bonding and molecular interactions further supported these findings. Most compounds had 5–6 hydrogen-bond donors and 2–3 acceptors, suggesting the possibility of multiple stabilising interactions with amino-acid residues in the catalytic site of LTA4H. Notably, Withanolide L had an ideal hydrogen-bond donor–acceptor balance (5 and 2, respectively) together with the strongest binding energy, indicating a favourable orientation and pose stability [14,23]. RMSD values supported the

stability of docked poses; most compounds showed values below 10 Å, with the few outliers (Sominone and Viscosalactone B, ~30 Å) likely reflecting the flexibility of withanolide side chains [24].

The drug-likeness profiles, assessed through Lipinski's Rule of Five, revealed that almost all compounds adhered strictly to the criteria. The molecular weights ranged between 452 and 488 g/mol, falling within the acceptable range for drug candidates. The lipophilicity (iLogP) of all compounds was moderate (3.21 to 3.69), conducive to membrane permeability and bioavailability without compromising solubility [20,25].

The ADME profile, predicted using SwissADME [21], confirmed acceptable gastrointestinal absorption and minimal violation of bioavailability rules. The BOILED-Egg model within SwissADME indicated good predicted intestinal absorption and low blood-brain barrier permeability for the lead withanolides, which is consistent with the desired distribution profile for a systemic, non-CNS-targeted anti-leukemic agent.

Target-prediction data from SwissTargetPrediction [22] aligned well with known cancer-related pathways. A significant number of compounds, especially the top binders, were predicted to interact with Protein Kinase C alpha (PKCα), an isoform implicated in leukemia pathogenesis [12]. This supports a dual mechanism in which the compounds may not only inhibit LTA4H but also modulate kinase pathways involved in leukemic cell proliferation and survival. Compounds such as Withanolide L, Withaferin A and Withanolide Q are particularly noteworthy in this context, as their predicted targets include PKC, which lies upstream of the MAPK/ERK and PI3K/AKT signalling cascades- both pathways essential for leukemic cell growth [10,11].

It is interesting to observe that while LTA4H was chosen as the primary docking target (6TJU), the SwissTargetPrediction results suggest that many of the compounds may also act on parallel or downstream targets such as Neprilysin, PKC and PKCα, reinforcing the pleiotropic nature of these phytochemicals [6,8]. This makes them particularly suitable for multifactorial diseases like leukemia, where multiple signalling pathways are dysregulated.

Notably, Somniferanolide and Sominone also showed strong binding affinities and were predicted to inhibit both LTA4H and Neprilysin (another zinc-dependent metallopeptidase involved in inflammation and cancer progression). This dual-inhibition potential makes them strong candidates for future development as multi-target anti-leukemic agents [4,23].

Furthermore, compounds such as Withaferin A- which is extensively studied in the literature for anti-cancer properties- also displayed excellent binding affinity (-9.0 kcal/mol) and drug-likeness, thereby validating the predictive reliability of the present study [3,10,13].

Taken together, the results of this study strongly suggest that *Withania somnifera* (L.), constituents-especially Withanolide L, Somniferanolide and Sominone- hold promise as inhibitors of LTA4H and modulators of oncogenic kinases [4,14]. Their excellent docking scores, favourable predicted ADME profiles and converging target-prediction data provide a strong foundation for subsequent *in vitro* validation.

5. CONCLUSION

The present *in-silico* investigation offers compelling evidence supporting the anti-leukemic potential of selected phytochemicals derived from *Withania somnifera* (L.), through inhibition of the Leukotriene A4 Hydrolase (LTA4H) enzyme. By utilising integrated computational methods- molecular docking via PyRx 0.8 [18,19], compound retrieval from PubChem and IMPPAT, pharmacokinetic / drug-likeness profiling through SwissADME [21] and target prediction through SwissTargetPrediction [22]- the study successfully identified a series of lead compounds with favourable pharmacodynamic and pharmacokinetic profiles.

Among the eleven phytochemicals studied, Withanolide L (-9.8 kcal/mol) emerged as the most potent binder to the 6TJU protein, suggesting a strong inhibitory potential against LTA4H. This was followed closely by Somniferanolide, Sominone, Withanolide Q and Withanolide R, each displaying binding energies of -9.3 kcal/mol or stronger. These findings indicate a strong likelihood that these compounds could interfere with LTA4H-mediated pathways that contribute to inflammation-driven leukemogenesis [2,4,14].

Almost all compounds fulfilled the essential criteria set by Lipinski's Rule of Five, including molecular weight (< 500 g/mol), number of hydrogen-bond donors and acceptors and acceptable lipophilicity (iLogP 3.21–3.69) [20]. The absence of significant Lipinski violations supports their predicted oral bioavailability. The RMSD values of the top-ranked docking poses remained within acceptable ranges (1.8–3.8 Å), further supporting the binding stability and specificity [24].

A key strength of this study lies in the convergent target-prediction outcome from SwissTargetPrediction [22], which highlighted Protein Kinase C alpha and Neprilysin as additional probable biological targets for several compounds. These proteins are known to participate in signal-transduction pathways that are dysregulated in leukemia [12]. Therefore, in addition to LTA4H inhibition, these phytochemicals may exert anti-leukemic effects through modulation of multiple oncogenic pathways, offering a polypharmacological approach to therapy.

Importantly, well-characterized compounds like Withaferin A and Withanolide Q- already recognised in the literature for their anti-cancer effects [3,6,10], also performed strongly in this study, validating the reliability of the *in-silico* model. Meanwhile, lesser-known compounds such as Sominone and Somniferanolide showed similar or better profiles, encouraging further exploration and possible isolation from plant material or synthetic derivatisation [8,16].

The implications of these findings are manifold:

- The compounds studied may serve as templates for the design of new anti-leukemic drugs.
- They offer potential leads for combination therapies, especially in cases of drug resistance.
- Their predicted pharmacokinetic profiles support oral administration, which may improve patient compliance.

However, while *in-silico* predictions are a powerful tool for narrowing down drug candidates, experimental validation is indispensable [15,17]. Future studies should include:

- *In vitro* cytotoxicity assays on leukemic cell lines (e.g., K562, HL-60, MOLT-4, THP-1) to confirm anti-proliferative activity
- Enzyme-inhibition assays to directly assess LTA4H inhibitory activity
- *In-vivo animal studies for pharmacokinetics and toxicity (proposed as a separate, follow-up programme of work)*
- Formulation development for delivery enhancement

In conclusion, this study establishes a promising computational framework for identifying novel anti-leukemic agents from traditional Ayurvedic sources [5,7,8]. *Withania somnifera* (L.), long regarded as a Rasayana in Ayurveda, continues to justify its therapeutic significance even in modern drug-discovery platforms [7,9]. The top-ranked compounds- especially Withanolide L, Somniferanolide and Sominone- demonstrate excellent binding affinity, predicted pharmacokinetic feasibility and mechanistic relevance, making them viable candidates for further development as anti-leukemic leads [4,14,23].

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