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Integrated computational screening of *Ageratina adenophora* metabolites for anticancer activity via ADMET, molecular docking, and cytotoxicity predictions

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Abstract

Ageratina adenophora is a plant rich in secondary metabolites with potential pharmacological applications. In this study, GC-MS and UHPLC-QTOF-MS analyses revealed a diverse array of phytochemicals, including sesquiterpenes, flavonoids, alkaloids, saponins, coumarins, sterols, and phenolic acids, highlighting the species as a promising source of bioactive compounds. To evaluate their therapeutic potential, an integrated in silico screening pipeline was employed, incorporating physicochemical characterization, drug-likeness evaluation, and ADMET profiling. Among the identified metabolites, Acon1_001440 emerged as the most promising candidate, exhibiting favorable pharmacokinetic properties, minimal PAINS alerts, and compliance with multiple drug-likeness filters. Molecular docking against key cancer-associated targets - EGFR, p53, MMP7, and CDK8/Cyclin C - revealed strong binding affinities of up to -8.2 kcal/mol, along with stable non-covalent interactions. Molecular dynamics simulations of the EGFR - Acon1_001440 complex further confirmed structural stability through consistent RMSD, RMSF, radius of gyration, solvent-accessible surface area (SASA), and hydrogen-bonding patterns. MM/GBSA binding free energy calculations (-12.28 kcal/mol) supported favorable binding energetics and suggested multitarget anticancer potential. Toxicity predictions indicated low acute oral toxicity, although potential carcinogenicity signals warrant further experimental validation. Collectively, these findings identify *A. adenophora* as a valuable reservoir of pharmacologically active metabolites and highlight Acon1_001440 as a promising lead compound for anticancer drug discovery. The integrated computational framework presented here provides a basis for future experimental validation and the development of multitarget anticancer agents.

Keywords: *Ageratina adenophora*, molecular docking, cytotoxicity, in silico, pharmacokinetics

1. Introduction

For centuries, plant-derived medicines have played a central role in human healthcare, partly due to concerns regarding the safety, tolerability, and adverse effects of synthetic pharmaceuticals^{1,2}. In recent decades, the use of natural remedies has increased, as plant-based therapies are often perceived as eco-friendly and potentially associated with fewer or more manageable side effects than conventional drugs^{2,3}. Most natural medicines

originate from crude plant extracts containing diverse phytoconstituents that contribute to a wide range of biological activities. Despite this reliance, only a fraction of the approximately 250,000 known plant species have undergone phytochemical screening, indicating substantial potential for the discovery of novel therapeutic agents^{2,4,5}.

Plants produce a complex system of secondary metabolites with diverse pharmacological properties, including antibacterial, antifungal, anti-inflammatory, antioxidant, and anticancer activities^{2,6}. These metabolites are commonly classified into phenolics, terpenoids, isoprenoids, and nitrogen- or sulfur-containing compounds, among others⁷. Despite their recognized therapeutic relevance, systematic characterization of plant-derived metabolites remains limited^{6,8}.

Ageratina adenophora (Crofton weed) is a perennial semi-herbaceous species belonging to the family Asteraceae. It can grow up to approximately two meters in height and is characterized by deep green, finely pubescent leaves often tinged with purple⁹. While considered invasive in many regions, *A. adenophora* contains diverse pharmacologically active metabolites, including terpenoids, flavonoids, alkaloids, saponins, coumarins, sterols, and phenolic acids. These compounds have been associated with antibacterial, antifungal, antiviral, anti-inflammatory, and antitumor activities¹⁰. In traditional medicine, the plant has been used to treat skin infections and fungal diseases, indicating ethnopharmacological relevance¹¹. However, toxicity concerns persist, and further pharmacological investigation is required to clarify its therapeutic potential and safety profile¹¹.

In silico approaches, including computer-aided drug design (CADD), have become important tools in early-stage drug discovery. These methods enable rapid screening of natural metabolites by predicting pharmacokinetic properties, drug-likeness, and target-binding affinities, reducing reliance on extensive experimental assays in the initial phases¹². Pharmacophore modeling and molecular docking provide insights into ligand–receptor interactions and support hypotheses regarding mechanisms of action, particularly when integrated with phytochemical profiling¹³. In this study, therapeutic targets were selected based on their established relevance in cancer-related signaling pathways and the availability of high-resolution structural data, given the limited experimental data available for *A. adenophora*-derived compounds.

Here, we profiled bioactive compounds from ethanol leaf extracts of *A. adenophora* using GC-MS and UHPLC-QTOF-MS. Identified metabolites were evaluated through molecular docking and molecular dynamics simulations to assess binding stability and target interactions, alongside in silico pharmacokinetic and ADMET predictions. In silico cytotoxicity analyses were further conducted across tumor and non-tumor cellular models. Together, these analyses integrate phytochemical profiling with computational pharmacology to assess the anticancer potential of *A. adenophora*-derived compounds.

2. Materials and methods

2.1. Compliance with ethical standards

This research was conducted following all relevant institutional, national, and international guidelines for plant research. Procedures were consistent with the IUCN Policy Statement on Research Involving Species at Risk of Extinction (Version 1.0, 1989)¹⁴ and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 1973, including current appendices)¹⁵. *A. adenophora* is classified as Least Concern according to the IUCN Red List (version 3.1, 2022)¹⁴ and is not included in any CITES appendices. No endangered or protected plant species were collected or used in this study.

2.2. Plant collection and processing

Leaves of *A. adenophora* were collected at Sta. Fe, Nueva Vizcaya, Philippines with prior approval from the local government and the Department of Environment and Natural Resources (DENR), at GPS coordinates 16°11'05.16"N, 120°54'48.69"E, at an elevation of 985 m above sea level. Taxonomic identification was verified by Mr. Paul Henric Gojo Cruz (Department of Biological Sciences, Central Luzon State University,

Nueva Ecija, Philippines). A voucher specimen (ID: EP0002) was deposited in the department herbarium for future reference.

Fresh, mature, and healthy leaves were collected in the early morning, placed in sterile resealable polyethylene bags, and transported to the laboratory. Upon arrival, leaves were rinsed thoroughly under running tap water to remove dirt, followed by distilled water. Surface sterilization was performed with 70% ethanol. Leaves were air-dried in the shade at ambient temperature (25 ± 2 °C) for 15 days. The dried material was ground into fine powder using a mechanical grinder, then stored in sterile airtight containers (1000 mL) at room temperature until extraction.

2.3. Ethanol extraction

Fifty grams of dried leaf powder were macerated in 500 mL of 95% ethanol at room temperature (25 ± 2 °C) in amber glass flasks for 72 h with intermittent shaking. The extract was filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40 °C using a rotary evaporator (IKA RV 10/HB 10, 220–240 rpm). The crude extract was centrifuged at $10,000 \times g$ for 30 min, then sterilized via a 0.22- μ m syringe filter (Acrodisc® 25 mm). Sterile extracts were stored at 4 °C until further analysis.

2.4. Derivatization and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

Samples (aliquots) were dried in a vacuum centrifuge at 25 °C for 90 min. Residues were oxidized by adding 20 μ L of methoxyamine hydrochloride in pyridine (30 mg/mL) and incubated at 37 °C for 2 h with intermittent mixing. Then, 20 μ L of BSTFA containing 1% TMCS was added, and incubation continued at 37 °C for 1 h to achieve trimethylsilylation. Samples were equilibrated at room temperature for 1 h before GC-MS analysis. Reagent blanks were prepared and processed in parallel.

GC-MS was conducted on an Agilent 8890 gas chromatograph coupled with a 5977B mass selective detector (Agilent Technologies, Santa Clara, CA, USA) using an HP-5MS capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness). Helium served as the carrier gas at a constant flow rate of 1 mL/min. One microliter of each sample was injected in split mode (10:1) at 300 °C. The oven program started at 45 °C (no hold), ramped to 300 °C at 10 °C/min, and was held for 9 min (total runtime 34.5 min). Mass spectrometry was performed in electron ionization (EI) mode at 70 eV with a scan range of m/z 50–550 and a scan time of 0.2s.

Tentative compound identification was based on retention times, peak areas, and mass fragmentation patterns compared to the NIST MS Search 2.3 library. Confidence was assessed using the Match Factor (MF) and Reverse Match Factor (RMF). MF values >900 were excellent, 800–900 good, 700–800 fair, and <600 poor. RMF complemented MF to reduce interference from ions absent in the reference spectra ¹⁶.

2.5. Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight-Mass Spectrometry (UHPLC-QTOF-MS) analysis

Metabolite profiling was performed using an Agilent 1260 Infinity II UHPLC coupled to a 6530 QTOF-MS/MS detector (Agilent Technologies, Santa Clara, CA, USA). Data were acquired in negative electrospray ionization (ESI⁻) mode across m/z 25–1000 at 5 spectra/s with an acquisition time of 200 ms per spectrum. Chromatographic separation used a Poroshell 120 EC-C18 column (4.6 $\times$ 150 mm, 4 μ m). The mobile phase was water (A) and methanol (B) at a constant flow rate and gradient program.

For sample prep, 250 μ L methanol was added to 2 mL microcentrifuge tubes containing the extract. Samples were vortexed for 1 min, sonicated for 5 min, and centrifuged at 12,000 rpm for 5 min. Fifty microliters of the supernatant were diluted with 250 μ L water and 250 μ L methanol, vortexed 1 min, sonicated 5 min, centrifuged at 1,200 rpm for 5 min, and transferred to autosampler vials.

Raw data were processed using Agilent MassHunter Qualitative Analysis Navigator (vB.08.00). Putative annotations were performed using spectral matching and molecular networking on the Global Natural Products

Social Molecular Networking (GNPS) platform. Cosine similarity was used to evaluate spectral alignment and compound similarity¹⁷.

2.6. ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) prediction

Phytochemicals prioritized for ADMET prediction were selected based on relative abundance, spectral annotation confidence ($\geq 80\%$ cosine similarity), and structural relevance to known bioactive scaffolds^{18,19}. Molecular structures and SMILES were retrieved from PubChem (<https://pubchem.ncbi.gov>, accessed October 2025). ADMET properties were predicted using Chemicalize® (<https://chemicalize.com> accessed October 2025) and SwissADME (<http://www.swissadme.ch> accessed October 2025) to evaluate pharmacokinetics, drug-likeness, and compliance with medicinal chemistry rules. Toxicity endpoints, including oral toxicity, hepatotoxicity, cytotoxicity, mutagenicity, carcinogenicity, and immunotoxicity, were assessed using ProTox-II (<https://tox.charite.de/protox3> accessed October 2025). Predictions were considered indicative rather than definitive^{18,20,21}.

2.7. Molecular docking

2.7.1 Selection of target proteins

Target proteins were selected for molecular docking based on their well-established roles in cancer progression, metastasis, and transcriptional regulation. The selected receptors included the epidermal growth factor receptor (EGFR), the tumor suppressor protein p53, matrix metalloproteinase-7 (MMP-7), and cyclin-dependent kinase 8 in complex with Cyclin C (CDK8/Cyclin C). EGFR, particularly its tyrosine kinase domain, plays a crucial role in the pathogenesis of non-small cell lung cancer and serves as a validated therapeutic target for tyrosine kinase inhibitors. Aberrant activation of EGFR signalling promotes uncontrolled cell proliferation and survival²². The transcription factor p53 is a central regulator of cell-cycle arrest, apoptosis, and genomic stability. Mutations in the TP53 gene impair its tumor suppressor function and are frequently associated with tumor progression²³. MMP-7, a member of the matrix metalloproteinase family, facilitates tumor invasion and metastasis through the degradation of extracellular matrix components²⁴. CDK8, in complex with Cyclin C, functions as a transcriptional co-regulator within oncogenic signalling pathways, including Wnt/ β -catenin and p53 signaling, and its dysregulation has been implicated in multiple malignancies²⁵.

2.7.2 Ligand preparation

Candidate ligands were selected from phytochemical constituents of *A. adenophora* based on two screening criteria: (i) predicted binding energies ≤ -6.0 kcal/mol, and (ii) compliance with drug-likeness filters, including the Lipinski rule of five and Veber, Ghose, Egan, and Muegge criteria²⁶. From a total of 126 tentatively identified volatile and non-volatile metabolites characterized by GC-MS and UHPLC-QTOF-MS analyses (Tables 1 and 2), six compounds were shortlisted for docking studies. The three-dimensional chemical structures were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>, accessed October 2025) in a structure data file (SDF) format. The selected ligand and their corresponding PubChem Compound Identifiers (CIDs) were as follows: (A) Usnic Acid (CID:5646); (B) Tryptophan (CID 6305); (C) 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl(Z)-2-methylbut-2-enoate (CID: 51136479) (D) Feruloyltyramine (CID: 5280537) (E) Acon1_001440 (CID: 23902325) (F) Pyrenophorol (CID 10935870). Erlotinib, a clinically approved EGFR inhibitor²², and eprentapopt (APR-246), a small molecule p53 reactivator²⁷, were included as positive controls for EGFR and TP53 docking studies, respectively.

The ligand preparation was conducted using PyRx Software, which incorporates the Open Babel module. All ligands were energy-minimized using the MMFF94 force field to optimize molecular geometry and assign partial charges. The minimized ligands were converted into PDBQT format, which is required for AutoDock-based docking simulations²⁸.

The three-dimensional crystal structure of the selected target proteins was retrieved from the Research Collaboratory of Structural Bioinformatics Protein Data Bank (RCSB PDB) (<https://www.rcsb.org/>, accessed 10 December 2025). The corresponding PDB identifier and crystallographic parameters were as follows: EGFR (PDB ID: 4LQM, resolution 2.5 Å); p53 (PDB ID 4MZ1, resolution 1.25Å); MMP-7 (PDB ID: 276D, resolution 1.60 Å); and CDK8/Cyclin C (PDB ID: resolution 2.45Å). All structures were derived from *Homo sapiens* and represent experimentally determined models with acceptable refinement statistics, including R-free and R-work values indicative of high structural quality.

2.7.3 Protein preparation and validation

Protein structures were visualized and inspected using PyMOL (version 3.1) to assess structural completeness and chain integrity²⁹. Protein preparation was carried out using Biovia Discovery Studio- 2025 Client³⁰. All non-protein entities, including crystallographic water molecules, metal ions, and co-crystallized ligand, were removed to prevent interference with ligand binding during docking simulation. Hydrogen atoms were added, and protonation states were adjusted to physiological pH (7.0) using the PROPKA algorithm to ensure appropriate ionization of amino acid residues.^{31–33} Partial atomic charges were assigned to provide an accurate electrostatic representation of the protein structures³⁴. The prepared protein receptors were subsequently converted and saved in PDBQT format for performing molecular docking analysis. The structural quality assessment was performed using Ramachandran plots analysis generated via the PROCHECK server integrated within the PDBsum database (<https://www.ebi.ac.uk/thornton-srv/databases/pdbsum>, accessed on 12 December 2025)²². The distribution of ϕ (phi) and ψ (psi) torsion angles was evaluated to confirm that the majority of amino acid residues occupied favored and allowed regions, indicating acceptable stereochemical quality of the prepared protein models.

2.7.4. Molecular docking protocol

Molecular docking is a key structure-based computational approach used to predict the preferred ligand binding orientation and binding affinities of small molecules within the active sites of macromolecular targets³⁵. In this study, molecular docking was employed to evaluate the interaction potential of selected phytochemical compounds against cancer-related protein targets through virtual screening and site-specific analyses. All ligand structures were geometry-optimized prior to docking using the Merck molecular force field 94 to obtain energetically favorable conformations³⁶. The prepared ligands were converted to the appropriate file format and assigned rotatable bonds to preserve torsional flexibility during docking simulations.

Docking grids were defined based on the experimentally validated active sites of each target protein, ensuring that ligand binding was restricted to biologically relevant regions, particularly ATP-binding or catalytic pockets. Grid box centers and dimensions were set as follows EGFR (PDB ID: 4LQM) with center coordinates X: –59.05, Y: –8.27, Z: –24.77; and grid dimensions X: 48.46 Å, Y: 65.48 Å, Z: 53.88 Å), tumor suppressor protein p53 (PDB ID: 4MZ1) with center X: –12.26, Y: –37.69, Z: 3.10 and dimensions X: 42.95 Å, Y: 50.94 Å, Z: 67.71 Å), MMP7 (PDB ID: 2Y6D) with center X: –25.45, Y: 8.98, Z: 9.06 and dimensions X: 54.23 Å, Y: 37.77 Å, Z: 51.27 Å), and CDK8/Cyclin C (PDB ID: 6T41) with center X: 4.04, Y: –10.23, Z: 15.20 and dimensions X: 81.89 Å, Y: 58.78 Å, Z: 52.56 Å). These parameters ensured comprehensive coverage of the functional binding cavities of each target protein.

Site-specific molecular docking was conducted using PyRx version 0.8, which integrates the Autodock Vina docking engine for efficient ligand-protein interaction prediction¹⁶. Default vina scoring parameters were applied, and ligands were docked with full conformational flexibility, while protein structures were treated as rigid. For each ligand-protein pair, multiple binding poses were generated, and the conformation with the lowest binding free energy was selected for further analysis. The docked complexes were visualized and analyzed using PyMol (version 2.0) and Biovia Discovery Studio Client (version 25). Interaction analyses focused on identifying hydrogen bonds, hydrophobic interactions, π - π stacking, and other non-covalent contacts contributing to binding stability and specificity^{16,37}.

2.7.5. Docking validation

To validate the reliability and accuracy of the docking protocol, a re-docking procedure was performed. The co-crystallized ligands were extracted from each protein structure and re-docked into their respective binding sites using the same docking parameters³⁸. The structural alignment between the re-docked poses and the original crystallographic conformation was assessed by calculating the root-mean-square deviation (RMSD) using the RDKit. An RMSD value of ≤ 2.0 Å was considered acceptable, indicating that the docking protocol could reliably reproduce experimentally observed ligand binding orientations.

2.7.6. Protein-ligand interaction analysis

Protein-ligand interactions were analyzed using BIOVIA Discovery Studio (version 25)³⁹. Post-docking interaction profiling focused on identifying key non-covalent interactions, including hydrogen bonds, hydrophobic (π - π -stacking and π - π alkyl interactions), and electrostatic forces⁴⁰. Interacting residues, hydrogen bond distances, and donor-hydrogen-acceptor (D-H $\cdots$ A) bond angles were evaluated to assess binding modes and complex stability, with emphasis on hydrogen bond geometry, as an indicator of interaction strength.

2.8. Molecular dynamics simulation

The Acon1-001440, which satisfied the ADMET screening criteria and exhibited the highest docking binding affinity of -8.2 kcal/mol, was further evaluated using molecular dynamics (MD) simulations to investigate the stability of the binding conformation and persistence of intermolecular interactions under dynamic conditions. MD simulation of the EGFR-Acon1_001440 complex was performed using Gromacs (version 2020.4). To ensure reproducibility and assess simulation convergence, three independent simulations were conducted for 100 ns. The simulation was executed on the high-performance computing cluster of the Bioinformatics Resources and Applications Facility.

Protein parameters were assigned using the CHARMM36 all-atom force field, while ligand topologies were generated using the SwissParam server²². Each complex was placed in a dodecahedral simulation box, solvated with explicit TIP3P water molecules⁴¹ and neutralized with Na⁺ or Cl⁻ ions to achieve an ionic strength of 0.1M.

Energy minimization was performed using the steepest descent and conjugate gradient algorithms until convergence ($F_{\text{max}} < 1000 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{nm}^{-1}$). Systems were equilibrated sequentially under NVT conditions at 300 K using the velocity-rescaling thermostat, followed by NPT equilibration at 1 bar using Parrinello-Rahman barostat⁴². Long-range electrostatic interactions were calculated using the particle mesh Ewald (PME) method, and time integration was carried out using the Verlet leapfrog algorithm⁴³. Production simulations were performed for 100 ns, with atomic coordinates recorded every 10 ps for post-simulation analyses using an NVIDIA GeForce RTX 4070 GPU. Structural and energetic metrics reported in subsequent sections were derived exclusively from equilibrated trajectory frames.

Binding free energies (ΔG_{bind}) were estimated using the Molecular Mechanism Poisson-Boltzmann Surface Area (MM/PBSA) method in the gmx-MMPBSA tool, based on representative snapshots extracted from the MD trajectories. The binding free energy (ΔG_{bind}) was calculated as:

$$\Delta G_{\text{bind}} = \Delta G_{\text{RL}} - (\Delta G_{\text{R}} + \Delta G_{\text{L}})$$

where ΔG_{RL} , ΔG_{R} , and ΔG_{L} denote the free energies of the complex, receptor, and ligand, respectively³⁴.

Furthermore, trajectory-based analyses were carried out to evaluate the stability and flexibility of each system. The root-mean-square fluctuation (RMSF) quantified residue-level flexibility. The radius of gyration (Rg) was assessed to determine protein compactness during simulation, and the number of hydrogen bonds (H-bonds) between the receptor and ligand was monitored to evaluate interaction persistence and binding strength. These

analyses provide detailed insight into the dynamic behavior and conformational stability of the complexes under physical conditions.

Trajectory analyses were limited to metrics directly supporting the interpretation of docking and MM/PBSA results. These included root-mean-square fluctuation (RMSF) to assess residue-level flexibility, radius of gyration (R_g) to evaluate protein compactness, and intermolecular hydrogen bond analysis to monitor binding persistence over time.

2.9. In silico cytotoxicity prediction

The cytotoxic potential of bioactive compounds identified through GC-MS and UHPLC-QTOF-MS analyses was assessed using the In-silico Cell Line Cytotoxicity Predictor (CLC-Pred; <http://www.way2drug.com/Cell-line/>, accessed October 2025). This computational platform predicts qualitative cytotoxic responses of small molecules across panels of human tumor and non-tumor cell lines using structure-activity relationship (SAR) modeling. Predictions in CLC-Pred are generated via the Prediction of Activity Spectra for Substances (PASS) algorithm, which employs a leave-one-out cross-validation procedure trained on experimentally verified cytotoxicity datasets, achieving an overall predictive accuracy of approximately 96%.

For each compound-cell line combination, two probability scores were calculated: PA (probability of biological activity), and PI(probability of inactivity). Compounds were considered potentially cytotoxic when PA exceeded PI ($PA > PI$), reflecting a higher likelihood of inducing a cytotoxic response ⁴⁴.

The predicted cytotoxicity profiles were integrated as an additional computational screening layer, complementing the outcomes of molecular docking, molecular dynamics simulations, and ADMET-based pharmacokinetic and safety evaluations. This multi-tiered in silico approach allowed for the relative prioritization of compounds, highlighting those most likely to exhibit anticancer activity for further investigation.

3. Results

3.1 Phytochemical composition of *A. adenophora* using GC-MS and UHPLC-QTOF-MS

GC-MS analysis of the ethanolic extract of *A. adenophora* resulted in the tentative identification of sixty-two (62) volatile compounds (Table 1). The identified constituents belonged to diverse chemical classes, including sesquiterpenes, fatty acids, phenolic compounds, carbohydrates, sterols, triterpenes, alkaloids, and isoprenoids, reflecting the chemical complexity of the extract. The chromatographic profile was dominated by 2,4,6-tri-tert-butyl-nitrobenzene, which appeared at multiple retention times and collectively accounted for 31.4135% of the total peak area. Among the terpenoid constituents, modephene (6.294%) was the most prominent sesquiterpene, followed by other sesquiterpenes such as caryophyllene, germacrene D, silphinene, and isocomene derivatives, which together contributed substantially to the volatile fraction of the extract. Fatty acids constituted a major group of detected metabolites. Palmitic acid (4.17%), α -Linolenic acid (2.54%), stearic acid (0.61%), arachidic acid (0.59%), and propionic acid (1.20%) were identified, indicating the presence of both saturated and unsaturated lipid components. Several phenolic acids and phenylpropanoid-related compounds were also identified, including ferulic acid (2.67%), isoferulic acid (1.20%), caffeic acid (2.61%), chlorogenic acid (0.69%), and 5-O-feruloylquinic acid (0.78%). Carbohydrates and sugar alcohols were prominently detected, with D-trehalose, D-glucose, myo-inositol (1.34%), and arabitol derivatives. High-molecular-weight lipophilic constituents were also observed, including squalene (1.35%), β -amyrin (1.33%), lupeol (2.83%), stigmasterol (1.92%), and ergosta-7,22-dien-3-ol (1.48%).

Peak	RT	MW	Tentative compound	%	MF	Nature of
1	9.304	117.15	L-Valine*	0.33	C ₅ H ₁₁ NO ₂	Amino acid
2	9.562	60.056	Urea*	0.33	CH ₄ N ₂ O	Organic acid
3	10.164	92.09	Glycerol*	3.16	C ₃ H ₈ O ₃	Carbohydrate
4	10.89	204.35	Silphiperfol-5-ene	0.60	C ₁₅ H ₂₄	Sesquiterpene

5	10.992	128.17	Naphthalene*	0.43	C ₁₀ H ₈	Naphthalene
6	11.181	204.35	Silphinene	0.85	C ₁₅ H ₂₄	Sesquiterpene
7	11.685	204.35	Modephene	4.31	C ₁₅ H ₂₄	Sesquiterpene
8	11.765	204.35	α -isocomene	1.04	C ₁₅ H ₂₄	Sesquiterpene
9	12.045	204.35	($\pm$)- β -isocomene	0.77	C ₁₅ H ₂₄	Sesquiterpene
10	12.177	204.35	Caryophyllene	0.89	C ₁₅ H ₂₄	Sesquiterpene
11	12.944	204.35	Germacrene D	0.77	C ₁₅ H ₂₄	Sesquiterpene
12	13.304	122.12	meso-erythritol*	0.25	C ₄ H ₁₀ O ₄	Carbohydrate
		122.12	L-threitol*	0.25	C ₄ H ₁₀ O ₄	Carbohydrate
		122.12	Erythritol*	0.25	C ₄ H ₁₀ O ₄	Carbohydrate
13	14.22	220.35	Caryophyllene oxide	0.54	C ₁₅ H ₂₄ O	Sesquiterpene
14	15.192	318.28	D-arabinose*	0.97	C ₁₅ H ₁₈ O ₉	Organic compound
15	15.828	152.15	D- (+)-arabitol*	0.57	C ₅ H ₁₂ O ₅	Carbohydrate
16	16.131	74.08	propanoic acid, 3-(2-naphthalenyloxy)-	1.85	C ₃ H ₆ O ₂	Fatty acid
17	16.268	308.41	17-Methoxy-4-methyl-d-homo-18norandrosta-4,8,13,15,17-pentaen-3-one	0.27	C ₂₁ H ₂₄ O ₂	Steroid
18	16.818	278.5	Neophytadiene	0.95	C ₂₀ H ₃₈	Terpenoid
19	16.869	169.18	isoquinoline*	2.01	C ₁₀ H ₇ N ₃	Alkaloid
20	16.921	228.37	Myristic acid*	0.29	C ₁₄ H ₂₈ O ₂	Fatty acid
21	17.127	291.4	2,4,6 Tri-tert-butylNitrobenzene	7.85	C ₁₈ H ₂₉ NO ₂	Phenolic
22	17.338	203.19	Quinic acid*	1.15	C ₁₁ H ₉ NO ₃	Quinoline
23	17.481	180.16	D-fructose*	1.21	C ₆ H ₁₂ O ₆	Carbohydrate
24	17.59	180.16	D-(-)-fructose*	1.21	C ₆ H ₁₂ O ₆	Carbohydrate
25	17.699	137.18	Tyramine*	0.32	C ₈ H ₁₁ NO	Phenethylamine
26	17.756	180.16	D-glucose*	0.60	C ₆ H ₁₂ O ₆	Carbohydrate
	17.756	180.16	D-allose*	0.60	C ₆ H ₁₂ O ₆	Carbohydrate
27	18.088	291.4	2,4,6 tri-tert-butylNitrobenzene	21.52	C ₁₈ H ₂₉ NO ₂	Aromatic nitro
28	18.351	180.16	Myoinositol *	1.34	C ₆ H ₁₂ O ₆	Organic oxygen
29	18.46	586.0	1Hbenzo[g]indazole*	1.78	C ₃₀ H ₂₈ CIN ₇ O ₄	Indazole
30	18.517	111.17	Thiazepine *	2.64	C ₅ H ₅ NS	Organic compound
31	18.877	256.42	Palmitic acid*	4.17	C ₁₆ H ₃₂ O ₂	Fatty acid
32	19.043	159.18	4-amino-naphthalen-1-ol*	0.43	C ₁₀ H ₉ NO	Amino acid
33	19.261	66.06	Propanedinitrile*	0.37	C ₃ H ₂ N ₂	Organonitrogen
	19.261	66.06	Propanoic acid*	1.20	C ₃ H ₆ O ₂	Fatty acid
34	19.392	194.18	Isoferulic acid*	1.20	C ₁₀ H ₁₀ O ₄	Phenolic
	19.392	194.18	Ferulic acid*	2.67	C ₁₀ H ₁₀ O ₄	Phenolic
35	19.816	180.16	Caffeic acid*	2.61	C ₉ H ₈ O ₄	Phenolic
36	20.073	296.5	Phytol*	0.67	C ₂₀ H ₄₀ O	Isoprenoid
37	20.176	177.16	5-hydroxyindole-2-carboxylic acid*	1.62	C ₉ H ₇ NO ₃	Aromatic compound
38	20.388	280.4	9,12-octadecadienoic acid (Z, Z)-*	2.54	C ₁₈ H ₃₂ O ₂	Unsaturated fatty
	20.388	280.4	9(E),11(E)-conjugated linoleic acid*			Unsaturated fatty
39	20.457	278.44	α -linolenic acid*	2.54	C ₁₈ H ₃₀ O ₂	Unsaturated fatty
40	20.628	284.5	Stearic acid*	0.61	C ₁₈ H ₃₆ O ₂	Fatty acid
41	20.714	135.13	9H-purin-2-amine	1.15	C ₅ H ₅ N ₅	Purine
42	22.128	236.22	α -d-glucopyranosiduronic acid*	0.56	C ₉ H ₁₆ O ₇	Lipid
43	22.179	136.15	Oxyacetophenone*	1.0	C ₈ H ₈ O ₂	Phenolic
44	22.271	312.5	Arachidic acid*	0.59	C ₂₀ H ₄₀ O ₂	Fatty acid
45	22.803	172.18	1,3-cyclohexanedicarboxylic acid*	0.24	C ₈ H ₁₂ O ₄	Carboxylic acid
46	23.100	247.33	Piperidine-4-carboxylic acid ethyl ester	0.43	C ₁₅ H ₂₁ NO ₂	Carboxylic acid
47	23.896	400.4	6-hydroxyflavone- β -D-glucoside*	0.28	C ₂₁ H ₂₀ O ₈	Carboxylic acid
48	23.936	342.30	D- (+)-turanose*	0.37	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
49	25.200	410.7	Squalene	1.35	C ₃₀ H ₅₀	Isoprenoid
50	25.984	342.30	D-trehalose*	0.26	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
51	26.597	342.30	D-trehalose*	0.38	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
52	26.722	342.30	D-trehalose*	0.36	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
53	26.722	342.30	Lactose*	0.36	C ₁₁ H ₂₂ O ₁₁	Carbohydrate
54	27.512	342.30	D- (+)-turanose*	0.28	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
	27.512	342.30	D-trehalose*	3.88	C ₁₂ H ₂₂ O ₁₁	Carbohydrate
55	27.621	368.3	5-O-feruloylquinic acid*	0.78	C ₁₇ C ₂₀ O ₉	Organic compound
	27.621	729.2	cis-5-O-feruloylquinic acid*	0.78	C ₃₂ H ₆₀ OSi ₅	Phenolic
56	27.867	354.31	Chlorogenic acid*	0.69	C ₁₆ H ₁₈ O ₉	Phenolic

57	28.445	190.24	Propyl 3-phenylacrylate	0.73	C ₁₂ H ₁₄ O ₂	Cinnamic acid ester
58	29.572	412.7	Stigmasterol*	1.92	C ₂₉ H ₄₈ O	Lipid
59	30.344	398.7	Ergosta-7,22-dien-3-ol, (3 β ,22E)-*	1.48	C ₂₈ H ₄₆ O	Sterol
60	30.568	426.7	β -amyrin*	1.33	C ₃₀ H ₅₀ O	Triterpene
61	32.725	426.7	Lupeol*	2.83	C ₃₀ H ₅₀ O	Triterpene
62	33.234	450.8	Phytyl decanoate	0.25	C ₃₀ H ₅₈ O ₂	Ester

*BSTFA with 1% TMCS

Table 1. The GC-MS analysis results of *A. adenophora* used in ADMET prediction.

UHPLC-QTOF-MS enabled the tentative identification of thirty-six (36) non-volatile compounds in the ethanolic extract of *A. adenophora* (Table 2). Compound annotation was based on accurate mass measurements, fragmentation patterns, and cosine similarity matching against reference databases. The detected metabolites represented multiple chemical classes, including coumarins, flavonoids, alkaloids, phenylpropanoids, fatty acids, polyketides, phospholipids, and phenylethanoids, indicating a chemically diverse extract. Coumarins constituted a prominent class including 6,7-dihydroxycoumarin-6-glucoside, 1-palmitoyl-2-hydroxy-sn-glycero-3-, 6,7-dihydroxycoumarin (esculetin) and structurally complex coumarin derivatives 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate. The flavonoid fraction was characterized by several glycosylated polyphenols, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, hyperoside, methoxy-myricetin-3-O-hexoside, and petunidin-3-O- β -glucopyranoside. Two structurally distinct alkaloids were tentatively identified namely 1-(5,10-dioxo-2,3,5a,6,7,8-hexahydro-1H-dipyrrolo[1,2-d:1',2'-f]pyrazin-10a-yl)propan-2-yl carbamate and 3-methyl-8,10,20,22-tetraoxa-3-azapentacyclo[15.7.0.0^{3,7}.0^{4,13}.0^{16,21}]tetracos-1(17),5,7(11),12,18,23-hexaen-14-one, contributing to the nitrogen-containing metabolite pool of the extract. The phenylpropanoid group was particularly well represented, comprising compounds associated with the shikimate and phenylpropanoid pathways. Identified metabolites included caffeic acid, 4-methoxycinnamic acid, 2-hydroxycinnamic acid, chlorogenic acid, 3-o-feruloylquinic acid, 3,4-di-o-caffeoylquinic acid, and feruloyltyramine, a complex phenylpropanoid glycoside. Several fatty acids and lipid-related metabolites were also detected, including azelaic acid, (10E,15E)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid, hydroxylated fatty acid derivatives, and glycerophospholipids such as 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine and 1-hexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol).

Peak No.	RT (min)	m/z	Tentative Compound	MW (g/mol)	MF	Cosine similarity	Chemical Nature
1	9.22	463.1	Quercetin-3-o-glucoside	464.4	C ₂₁ H ₂₀ O ₁₂	0.99	Flavonoid
2	6.27	339.1	6,7-dihydroxycoumarin-6-glucoside	340.3	C ₁₅ H ₁₆ O ₉	0.97	Coumarin
3	23.74	325.2	2,4-dihydroxyheptadec-16-ynyl acetate	326.5	C ₁₉ H ₃₄ O ₄	0.94	Fatty acid
4	9.49	179.0	Caffeic acid	180.2	C ₉ H ₈ O ₄	0.95	Phenylpropanoid
			16- hydroxyoalmitic acid	272.42	C ₁₆ H ₂₃ O ₃	0.94	Fatty acid
5	10.40	343.1	Usnic acid	344.3	C ₁₈ H ₁₆ O ₇	0.94	Polyketides
6	15.56	463.1	Quercetin-3-o-rutinoside	610.5	C ₂₇ H ₃₀ O ₁₆	0.94	Flavonoid
7	7.96	297.1	Decylbenzenesulfonic acid	298.4	C ₁₆ H ₂₆ O ₃ S	0.94	Polyketides
8	6.27	203.1	Tryptophan	204.2	C ₁₁ H ₁₂ N ₂ O ₂	0.94	Amino acid
9	9.49	177.1	4-methoxycinnamic acid	178.2	C ₁₀ H ₁₀ O ₃	0.94	Phenylpropanoid
10	21.59	452.3	1-palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	453.5	C ₂₁ H ₄₄ NO ₇ P	0.93	Coumarin
11	27.56	341.1	(8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-	342.3	C ₁₉ H ₁₈ O ₆	0.93	Coumarin
12	8.30	367.1	3-o-feruloylquinic acid	368.3		0.93	Phenylpropanoid
13	6.75	353.1	5-o-caffeoylquinic acid	354.3	C ₁₆ H ₁₈ O ₉	0.92	Phenylpropanoid

14	7.51	177.0	6,7-dihydroxycoumarin esculetin cichorigenin aesculetin 6,7-dihydroxy-2-benzopyrone esculetin cichoriin aglycon 6,7-Dihydroxy-2H-1-benzopyran-2-one esculin aglycon	178.1	C ₉ H ₆ O ₄	0.92	Coumarin
15	23.93	483.3	1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phospho-(1'-rac-glycerol)		C ₄₀ H ₇₇ O ₁₀ P	0.92	Fatty acid
16	7.95	515.1	3,4-di-o-caffeoylquinic acid	516.4	C ₂₅ H ₂₄ O ₁₂	0.92	Phenylpropanoid
17	1.41	163.0	2-hydroxycinnamic acid	164.2	C ₉ H ₈ O ₃	0.91	Phenylpropanoid
18	8.84	329.2	PR309108 FA 18:1+3O			0.91	Fatty acid
19	10.68	187.1	Azelaic acid	188.2	C ₉ H ₁₆ O ₄	0.91	Fatty acid
20	20.20	294.2	1-(5,10-dioxo-2,3,5a,6,7,8-hexahydro-1H-dipyrrolo[1,2-d:1',2'-f]pyrazin-10a-yl)propan-2-yl carbamate	295.3	C ₁₄ H ₂₁ N ₃ O ₄	0.90	Alkaloid
21	9.13	191	Quinate	192.1	C ₇ H ₁₂ O ₆	0.90	Phenolic acid
22	5.78	707.2	Chlorogenic acid	354.3	C ₁₆ H ₁₈ O ₉	0.90	Phenylpropanoid
23	6.75	137.0	Protocatechuic aldehyde	138.1	C ₇ H ₆ O ₃	0.89	Phenolic acid
24	10.74	433.1	[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-(4-hydroxyphenoxy) oxan-2-yl] methyl (E)-3-(3,4-dihydroxyphenyl)prop-2-enoate	434.4	C ₂₁ H ₂₂ O ₁₀	0.89	Phenylpropanoid
25	13.37		(10E,15E)-9,12,13-trihydroxyoctadeca-10,15-dienoic acid	328.4	C ₁₈ H ₃₂ O ₄	0.88	Fatty acid
26	9.26	493.1	methoxy-myricetin-3-o-hexoside	494.4	C ₂₂ H ₂₂ O ₁₃	0.88	Flavonoid
27	1.41	151.1	Divarinol	152.2	C ₉ H ₁₂ O ₂	0.88	Polyketides
28	15.94	477.1	Calceolarioside	478.4	C ₂₃ H ₂₆ O ₁₁	0.88	Phenylethanoid
29	23.93	483.3	1-hexadecanoyl-sn-glycero-3-phospho-(1'-myo-inositol)	572.6	C ₂₅ H ₄₉ O ₁₂ P	0.88	Glycerophospholipid
30	11.70	312.1	Feruloyl tyramine	313.3	C ₁₈ H ₁₉ NO ₄	0.88	Phenylpropanoid
31	9.21	464.1	Hyperoside	464.4	C ₂₁ H ₂₀ O ₁₂	0.86	Flavonoid
32	8.30	118.0	Amino malonic acid	119.1	C ₃ H ₅ NO ₄	0.85	Amino acid
33	6.27	507.3	1,2-dilinooleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)	771	C ₄₂ H ₇₅ O ₁₀ P	0.84	Phospholipid
34	27.56	427.2	Petunidin-3-o-beta-glucopyranoside Pt 3-Glc petunidin 3-glucoside (2S,3R,4S,5S,6R)-2-[2-(3,4-dihydroxy-5-methoxyphenyl)-5,7-dihydroxychromenylum-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	514.9	C ₉ H ₁₆ O ₄	0.83	Flavonoid
35	5.78	352.1	Acon1_001440 (3-methyl-8,10,20,22-tetraoxa-3-azapentacyclo [15.7.0.0.5,13,07,11.019,23] tetracosa-1(24),5,7(11),12,17,19(23)-hexaen-14-one)	353.4	C ₂₀ H ₁₉ NO ₅	0.82	Alkaloid
36	4.34	311.2	Pyrenophorol	312.4	C ₁₆ H ₂₄ O ₆	0.8	Polyketides

Table 2. *A. adenophora* compounds based on UHPLC-QTOF-MS analysis used in ADMET prediction.

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3.2 ADMET analysis

Six phytochemical constituents of *A. Adenophora* such as usnic acid (A), tryptophan (B), 8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate (C), feruloyl tyramine (D), Acon1_001440 (E), pyrenophorol (F) satisfied all five major drug-likeness criteria (Lipinski, Ghose, Veber, Egan, and Muegge) without violations (Table 3). The control drug erlotinib (H) and eprenetopopt (APR-246) (H) satisfied the drug likeness except for Ghose and Mugge for APR-246. All remaining compounds failed at least one of these filters and were therefore deprioritized for further ADMET evaluation.

The Abbot Bioavailability Score (ABS) predicted a moderate probability of oral bioavailability for all six compounds with values ranging from 0.55 to 0.56, consistent with their favorable physicochemical profiles. Although all satisfied the drug-likeness criteria, each displayed at least one lead-likeness violation, suggesting potential limitations related to molecular size and structural complexity. Furthermore, Brenk's structural alerts analysis identified potentially reactive groups in several compounds. Notably, Acon1_001440 (E) exhibited no Brenk alerts, indicating a comparatively cleaner chemical scaffold. In contrast, usnic acid (A), feruloyl tyramine (D), pyrenophorol (F) each showed one Brenk alert, whereas tryptophan (B) 8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate (C) showed two alerts, reflecting a higher likelihood of chemical liabilities.

Bioactive compound	A	B	C	D	E	F	G	H
ADME Properties								
Lipinski # violations	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0
Ghose # violations	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	No; 1
Veber # violations	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0
Egan # violations	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0
Muegge # violations	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	Yes; 0	No; 1
Bioavailability score	0.56	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Pan assay interference compounds (PAINS) # alerts	0	0	0	0	0	0	0	0
Brenk # alerts	1	2	2	1	0	1	1	0
Lead likeness # violations	0	0	0	0	1	0	2	1
Synthetic accessibility	4.19	4.09	4.09	2.55	3.26	4.9	3.19	3.56

(A) Usnic acid (B) Tryptophan (C) (8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate (D) Feruloyl tyramine (E) Acon1_001440 (F) Pyrenophorol (G) Erlotinib (H) Eprenetapopt (APR-246)

Table 3. Predicted drug-likeness results of the phytochemical compounds of *A. adenophora*.

Physicochemical descriptors of the six compounds are summarized in Tables 4 and 5. Molecular weights ranged from 204.23 to 344.32 g/mol, remaining well below the 500 g/mol threshold of Lipinski's rule of five. Consensus lipophilicity values (average Log P) ranged from 0.18 to 2.89, indicating moderate lipophilicity compatible with membrane permeability and oral absorption. Topological polar surface (TPSA), hydrogen-bond donor and acceptor counts, and molecular flexibility values were likewise within ranges considered favorable for orally bioavailable drug candidates.

Bioactive compound	A	B	C	D	E	F	G	H
ADME Properties								
Size (MW) g/mol	344.32	204.23	342.34	313.35	353.37	312.3	393.44	199.25

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Lipophilicity XLOGP3)	2.88	-1.06	3.2	2.1	2.62	1.43	3.31	-0.09
Solubility Log S (ESOL)	1.5	0.18	2.89	2.39	2.68	1.33	-4.11	-0.82
Saturation Fraction Csp3	0.33	0.18	0.32	0.17	0.35	0.62	0.27	0.9
Flexibility (Num, rotatable bonds)	2	3	3	7	0	0	10	3
Num. H-bond acceptors	7	3	6	4	6	6	6	4
Num. H-bond donors	2	3	0	3	0	2	1	1
Molar refractivity	86.54	57.36	91.55	88.98	97.49	80.86	111.4	55.25

(A) Usnic acid (B) Tryptophan (C) (8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate (D) Feruloyl tyramine (E) Acon1_001440 (F) Pyrenophorol (G) Erlotinib (H) Eprenetapopt (APR-246)

Table 4. Physiochemical properties and computational descriptors of the phytochemical compounds of *A. adenophora*.

Bioactive compounds	A	B	C	D	E	F	G	H
ADME Properties								
Polarity TPSA	117.97	79.11	82.81	78.79	57.23	93.06	74.73	49.77
XLOGP3	2.88	-1.06	3.2	2.1	2.62	1.43	3.31	-0.09
ILOGP	0.85	0.99	2.71	2.58	3.18	2.52	3.67	2.02
WLOGP	1.5	1.12	3.02	2.37	2.22	1.26	3.48	-0.72
MLOGP	-0.52	-1.66	1.76	1.89	1.9	0.95	1.48	-0.35
SILICOS-IT	2.8	1.53	3.76	3.01	3.47	0.48	4.06	0.84
Lipophilicity average	1.5	0.18	2.89	2.39	2.68	1.33	3.2	0.34

(A) Usnic acid (B) Tryptophan (C) (8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate (D) Feruloyl tyramine (E) Acon1_001440 (F) Pyrenophorol (G) Erlotinib (H) Eprenetapopt (APR-246)

Table 5. Lipophilicity results of the phytochemical compounds of *A. adenophora*.

Predicted pharmacokinetic properties (Table 6) indicated high gastrointestinal absorption for all six compounds, supporting their potential suitability for oral administration. Among the evaluated compounds, Acon1_001440 was uniquely predicted to be blood-brain barrier (BBB) permeable and a substrate of P-glycoprotein (P-gp), suggesting possible central nervous system exposure to susceptibility to efflux-mediated transport.

Cytochrome P450 (CYP) inhibition profiling revealed minimal metabolic liability for most compounds. Usnic acid (A), tryptophan (B), 8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate (C), feruloyl tyramine (D), pyrenophorol (F) were predicted to be non-inhibitors of all major CYP isoforms examined. In contrast, Acon1_001440 (E) exhibited predicted inhibitory activity against CYP1A2, CYP2C9, CYP2D6, and CYP3A4, with no inhibition of CYP2C1, indicating a higher potential for drug-drug interactions.

Bioactive compound	A	B	C	D	E	F	G	H
ADME Properties								
Gastrointestinal (GI) absorption	High	High	High	High	High	High	High	High
Blood-brain barrier (BBB) permeability	No	No	No	No	Yes	No	Yes	No
P-gp substrate	No	No	No	No	Yes	No	No	No
CYP1A2 inhibitor	No	No	No	No	Yes	No	Yes	No
CYP2C19 inhibitor	No	No	No	No	No	No	Yes	No
CYP2C9 inhibitor	No	No	No	No	Yes	No	Yes	No

CYP2D6 inhibitor	No	No	No	Yes	Yes	No	Yes	No
CYP3A4 inhibitor	No	No	No	Yes	Yes	No	Yes	No
Log Kp (skin permeation) cm/s	-6.39	-8.3	-6.12	-6.72	-6.6	-7.19	-6.35	-7.58

(A) Usnic acid (B) Tryptophan (C) (8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f] chromen-9-yl) (Z)-2-methylbut-2-enoate (D) Feruloyl tyramine (E) Acon1_001440 (F) Pyrenophorol (G) Erlotinib (H) Epremetapopt (APR-246)

Table 6. Predicted pharmacokinetics results of the phytochemical compounds of *A. adenophora*.

The predicted toxicological profiles of the selected compounds are summarized in Table 7. Acon_001440 (E) and pyrenophorol (F) showed LD₅₀ values exceeding 2000 mg kg⁻¹, indicating low acute oral toxicity (toxicity classes 5 and 4, respectively). Among the phytochemicals evaluated, tryptophan (B) displayed the most favorable safety profile, being predicted as non-hepatotoxic, non-carcinogenic, non-cytotoxic, and non-mutagenic, although it exhibited moderate acute toxicity (LD₅₀). In contrast, compounds, 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate (C), feruloyl tyramine (D), and pyrenophorol (F) were predicted to possess immunotoxic potential, suggesting the presence of functional moieties capable of modulating immune-related pathways. Additionally, usnic acid (A) and Acon1_001440 (E) were predicted to have carcinogenic potential. For comparison, the reference anticancer drug erlotinib displayed predicted hepatotoxic, cytotoxic, immunotoxic, and mutagenic properties, along with a low LD₅₀ (125 mg kg⁻¹), indicating higher acute toxicity. Conversely, epremetapopt (APR-246) was predicted to be inactive across all evaluated toxicity endpoints and exhibited a moderate LD₅₀ (900 mg kg⁻¹), reflecting a comparatively favorable safety profile.

No	Compound	Hepatotoxicity		Carcinogenicity		Cytotoxicity		Immunotoxicity		Mutagenicity		Predicted LD50 (mg kg ⁻¹)	Toxicity class
		Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb		
A	Usnic acid	Inactive	0.61	Active	0.57	Inactive	0.72	Inactive	0.51	Inactive	0.89	995	4
B	Tryptophan	Inactive	0.64	Inactive	0.91	Inactive	0.74	Inactive	0.99	Inactive	0.96	80	3
C	(8,8-dimethyl-2,10-dioxo-9H-pyrano [2,3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate	Inactive	0.63	Inactive	0.54	Inactive	0.67	Active	0.90	Inactive	0.54	832	4
D	Feruloyltyramine	Inactive	0.77	Inactive	0.57	Inactive	0.98	Active	0.94	Inactive	0.61	500	4
E	Acon1_0014440	Inactive	0.90	Active	0.61	Inactive	0.56	Inactive	0.56	Inactive	0.56	3200	5
F	Pyrenophorol	Inactive	0.76	Inactive	0.67	Inactive	0.57	Active	0.54	Inactive	0.83	5000	5
G	Erlotinib	Active	0.78	Inactive	0.51	Active	0.75	Active	0.91	Active	0.55	125	3
H	Epremetapopt (APR-246)	Inactive	0.93	Inactive	0.25	Inactive	0.75	Inactive	0.97	Inactive	0.73	900	4

*Pr: prediction; Pb: probability

Table 7. Toxicological properties of selected bioactive compounds of *A. adenophora*.

The bioavailability radar plot (Figure 1) summarizes the drug-likeness of the selected *A. adenophora* bioactive compounds based on six key physicochemical parameters: lipophilicity, molecular size, polarity, solubility, flexibility, and saturation. Usnic acid (A), 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (Z)-2-methylbut-2-enoate (C), Acon1_001440 (E), and pyrenophorol (F) are fully confined within the optimal (pink) radar region, indicating compliance with the recommended physicochemical space for oral bioavailability. In contrast, tryptophan (B), feruloyl tyramine (D), and reference drug erlotinib (G) and epremetapopt (H) deviated primarily due to polarity and low saturation values (fraction Csp³ > 0.25), reflecting lower three-dimensional complexity.

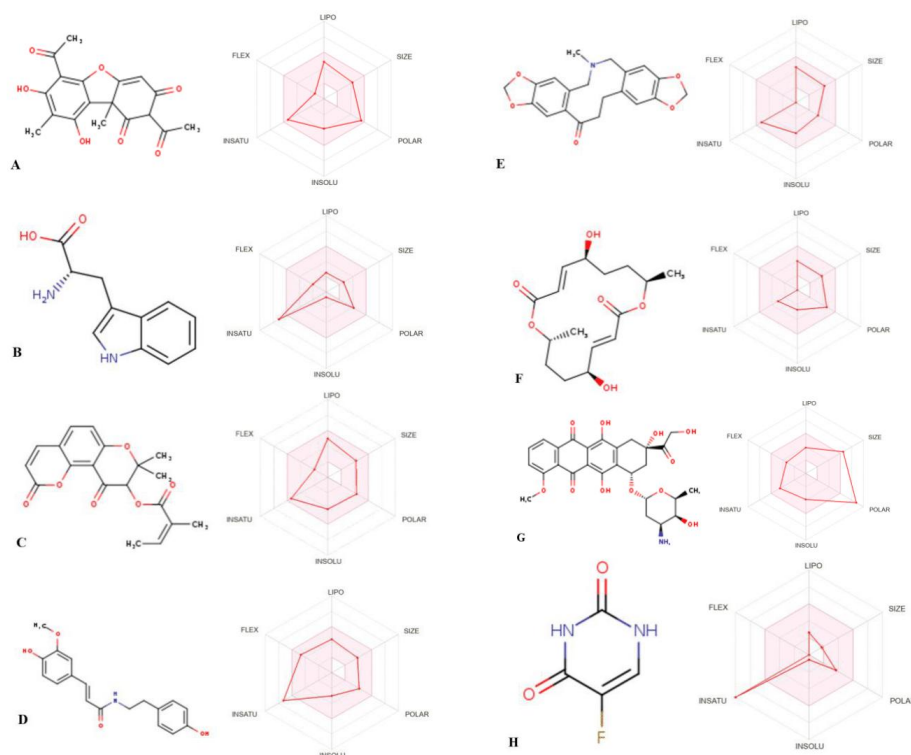


Figure 1. Bioavailability radar of the bioactive compounds of *A. adenophora* (A) usnic acid; (B) tryptophan (C) 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate; (D) feruloyltyramine; (E) Acon1_001440 (F) pyrenophorol (G) erlotinib (H) eprenapopt (ARG-246).

3.3 Molecular docking analysis

Six compounds that satisfied established drug-likeness criteria were included in the analysis: usnic acid (A), tryptophan (B), 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate (C), feruloyl tyramine D, Acon1_001440 (E), pyrenophorol (F). The three-dimensional structures of all ligands were retrieved from the PubChem database and prepared by geometry optimization and assignment of rotatable bonds before docking. Four cancer-associated protein targets were selected for docking analysis, such as EGFR (PD ID: 4lqm), tumor suppressor protein p53 (PD ID: 6ff9), MMP7 (PD ID: 2y6d), and CDK8/Cyclin C (PD ID: 6t41). Protein crystal structures were obtained from the Research Collaboratory of Structural Bioinformatics Protein Data Bank (RCSB PDB). All protein structures were pre-processed before docking. Crystallographic water molecules, heteroatoms, and non-essential chains were removed. Hydrogen atoms were added, and energy minimization was performed to relieve steric clashes and optimize protein geometry. Structure-based molecular docking was performed using AutoDock Vina implemented in PyRx. Docking grids were defined to encompass the active binding sites of each target protein. Binding affinity scores (kcal/mol) were used to rank ligand-protein interactions. Erlotinib and eprenapopt (APR-246) were included as reference ligands to provide comparative benchmarks for docking score interpretation. Ligand efficiency (LE) was calculated as the absolute value of binding energy normalized to the number of heavy atoms. Predicted inhibition constant (Ki) was estimated from docking scores, and hydrogen bond interactions were recorded for each docked complex. Docked complexes of the top-ranked ligand were visualized and analyzed using Discovery Studio Visualizer to identify key intermolecular interactions, including hydrogen bonding, hydrophobic contacts, van der Waals forces, and π -based interactions.

3.3.1 Protein structure validation

Ramachandran plots analysis showed that more than 98% of amino acid residues in EGFR, p53, MMP7, and CDK8/Cyclin C were located within the most favored and additionally allowed regions, with fewer than 2% of residues

in disallowed regions (Figure 2). This distribution indicates acceptable stereochemical quality and confirms that the prepared protein structures were suitable for molecular docking analyses.

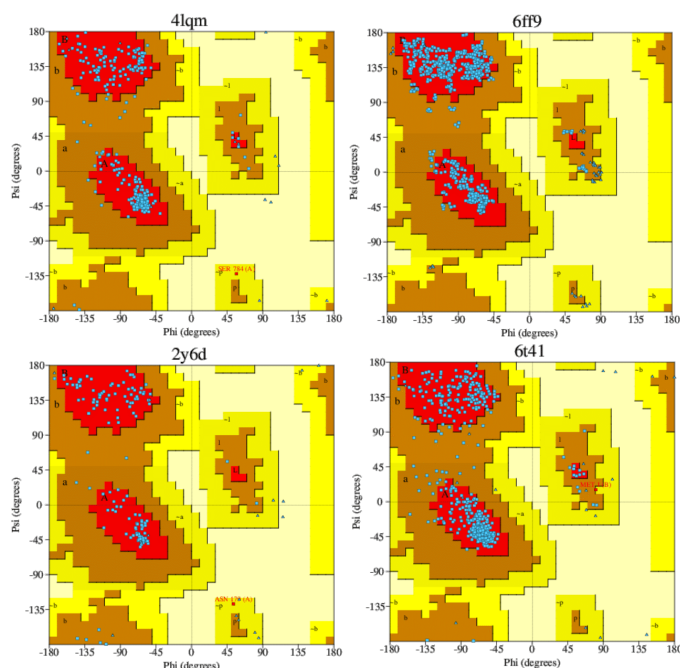


Figure. 2. Ramchandran plot analysis of EGFR (4lqm), p53 (6ff9), MMP7, and CDK8/Cyclin C (6t41).

3.3.2 Docking score and binding affinities

Docking results for all ligands across the four cancer-related targets are summarized in Table 8. Among the screened compounds, Acon1_001440 consistently exhibited one of the most favorable binding affinities across all targets. For EGFRF, Acon1_001440 showed the strongest predicted affinity (-8.2 kcal/mol), exceeding that of the reference ligand erlotinib (-7.6 kcal/mol) and eprenetapopt (-5.0 kcal/mol). For p53, Acon1_001440 exhibited a binding affinity of -6.6 kcal/mol, comparable to erlotinib (-6.2 kcal/mol) and stronger than eprenetapopt (-4.5 kcal/mol). For MMP7, Acon1_001440 again demonstrated a favorable docking score (-7.4 kcal/mol), closely matching erlotinib and outperforming eprenetapopt. The predicted interaction for Acon1_001440 was with CDK8/Cyclin C, with a binding energy of -8.1 kcal/mol, comparable to that of erlotinib (-7.9 kcal/mol).

3.3.3. Ligand efficiency and predicted inhibition constants

The binding modes of Acon_001440 with each target protein are illustrated in Figure 3- 7. In the EGFR complex, Acon1_001440 formed hydrogen bonds with Ile 1018 and Leu 703, supported by extensive van der Waals and hydrophobic interactions involving residues such as Leu 1017, Asp 770, Arg 776, and Pro 1019. Interaction with p53 involved hydrogen bonding with Thr 189, van der Waals contacts with surrounding residues, and a π - π T-shaped interaction with Phe 103. In the MMP7 complex, carbon-hydrogen bonding and hydrophobic interaction involving residues such as Tyr 215, Leu 181, and Trp 203 were observed. For CDK8/Cyclin C, Acon1_001440 established multiple hydrogen bonds with Arg 178, Ser 62, and Ala 177, along with stabilizing hydrophobic contacts.

The docking analysis indicates that Acon1_001440 exhibits a consistent and diverse interaction profile across multiple cancer-related protein targets, characterized by favorable binding energies, efficient size-normalized binding, and stable interaction networks.

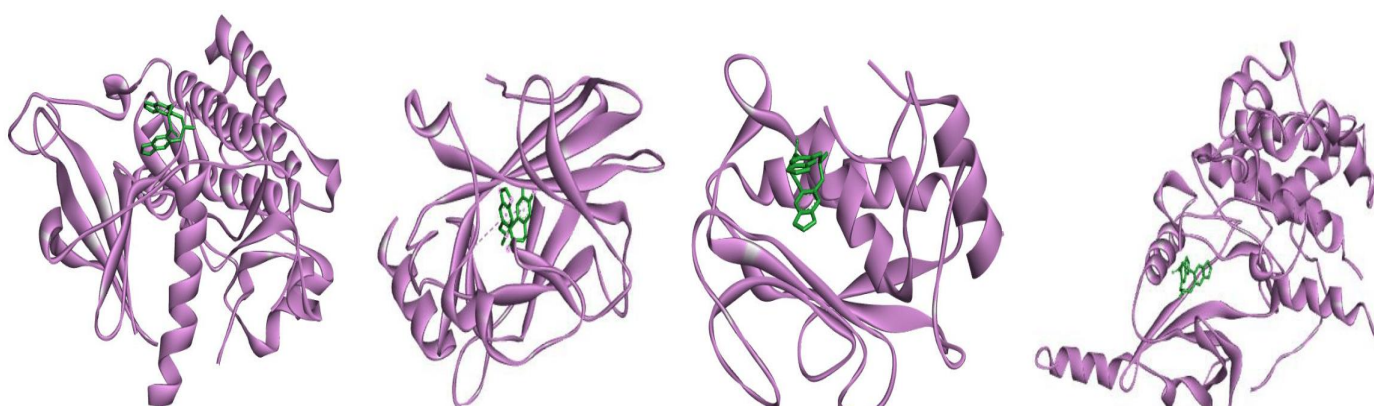


Figure 3. Docking poses of Acon1_001440 with EGFR, p53, MMP7, and CDK8/ Cyclin C.

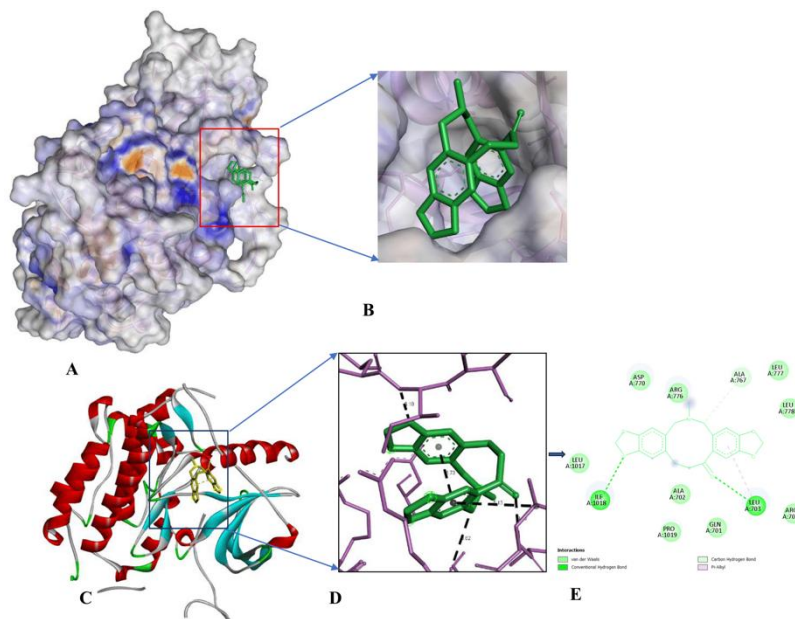


Figure 4. Docking Interaction of Acon 1_001440 with EGFR (4lqm). (A) Molecular surface of the docked complex (B) 3D hydrophobicity surface plot at the binding site (C) Overall ribbon structure (D) Ligand interaction with target protein (E) 2-D interaction of the complex

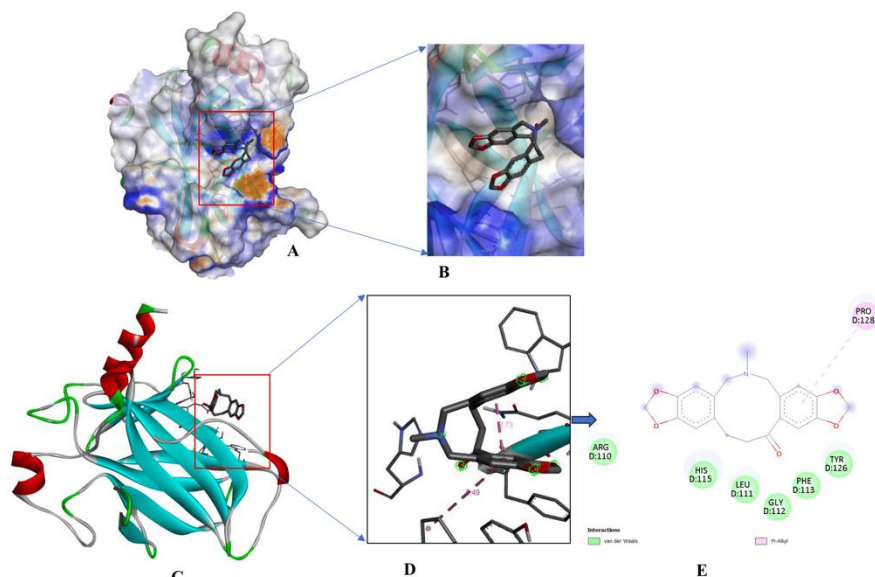


Figure 5. Docking Interaction of Acon 1_001440 with p53 (6ff9). (A) Molecular surface of the docked complex (B) 3D hydrophobicity surface plot at the binding site (C) Overall ribbon structure (D) Ligand interaction with target protein (E) 2-D interaction of the complex.

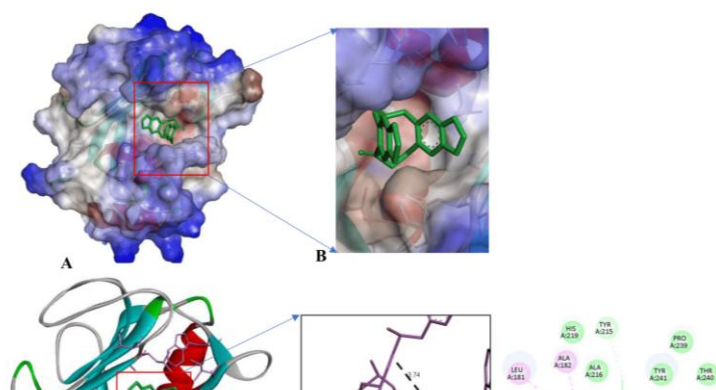


Figure 6. Docking Interaction of Acon 1_001440 with MMP7 (2y6d). (A) Molecular surface of the docked complex (B) 3D hydrophobicity surface plot at the binding site (C) Overall ribbon structure. (D) Ligand interaction with target protein. (E) 2-D interaction of the complex

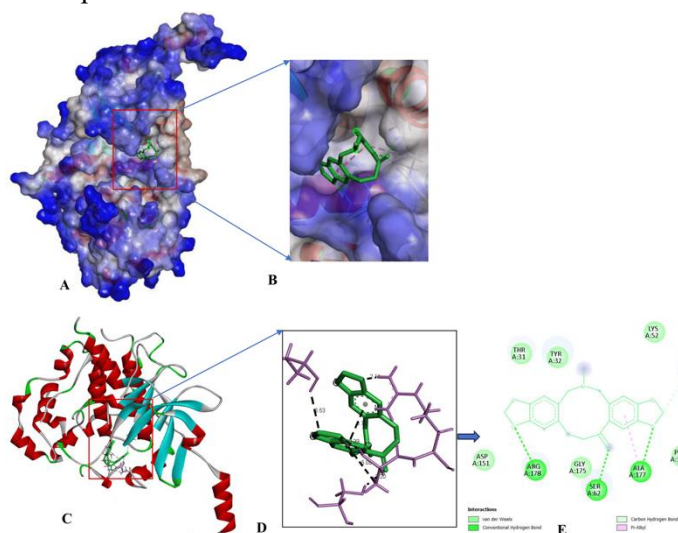


Figure 7. Docking Interaction of Acon 1_001440 with CDK8/Cyclin C (6t41). (A) Molecular surface of the docked complex (B) 3D hydrophobicity surface plot at the binding site (C) Overall ribbon structure (D) Ligand interaction with target protein (E) 2-D interaction of the complex

Code	EGFR (PD ID: 4lqm)					p53 (PD ID: 6ff9)				MMP7 (PD ID: 2y6d)				CDK8/Cyclin C (PD ID: 6t41)				No of H Bonds
	Ligand	Binding Energy	Ligand Efficiency	Inhibitory Constant (μM)	No of H Bonds	Binding Energy	Ligand Efficiency	Inhibitory Constant (μM)	No of H Bonds	Binding Energy	Ligand Efficiency	Inhibitory Constant (μM)	No of H Bonds	Binding Energy	Ligand Efficiency	Inhibitory Constant (μM)	No of H Bonds	
A	Usnic acid (CID: 5646)	-7.6	0.30	2.65	1	-6.6	0.26	1.43	1	-7.1	0.28	6.16	0	-8.9	0.36	2.94	3	624
B	Tryptophan (ID: 6305)	-6.7	0.44	1.21	4	-5.8	0.39	5.54	3	-7.5	0.5	3.13	4	-7.3	0.48	4.39	3	625
C	8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl) (Z)- 2-methylbut-2-enoate (CID: 51136479)	-7.7	0.30	2.24	0	-7.2	0.29	5.20	1	-8.2	0.33	9.60	0	-9.0	0.36	2.48	0	626
D	Feruloyl tyramine (CID: 5280537)	-7.3	0.31	4.39	3	-6.6	0.29	1.43	4	-8.4	0.37	6.84	2	-8.3	0.36	8.11	2	627
E	Acon1_001440 (CID: 23902325)	-8.2	0.32	9.60	2	-6.6	0.25	1.43	0	-7.4	0.28	3.71	0	-8.1	0.31	1.14	3	628
F	Pyrenophorol (CID:10935870)	-7.8	0.35	1.89	0	-6.1	0.28	3.33	1	-6.7	0.30	1.21	1	-7.3	0.33	4.39	1	629
G	Erlotinib (CID: 176870)	-7.6	0.76	2.65	2	-6.2	0.62	2.81	1	-8.2	0.82	9.60	1	-7.9	0.79	1.59	1	630
H	Eprenapopt (CID: 52918385)	-5.0	1-67	2.14	1	-4.5	1.5	< 1	1	-5.3	1.77	< 1	1	-5.6	1.87	7.77	0	631

Table 8. Docking-derived binding energies, ligand efficiencies (LE), predicted inhibition constant (Ki), and hydrogen bond interactions of A. adenophora bioactive compounds and reference ligand with cancer- related protein targets. energy of bioactive compounds with the target cancer-related protein receptors.

3.4. Molecular dynamics simulations of the EGFR- Acon1_001440complex

The best docked-ligand-protein complex was further analyzed for molecular dynamics simulation. Due to its superior pharmacological properties and favorable binding affinity, reflected by an estimated binding free energy of -8.2 kcal/mol, Acon1_001440 was selected for molecular dynamics (MD) simulation analysis to assess the stability and behavior of the Acon1_001440- EGFR complex. The binding energy value of -8.2 kcal/mol indicates a strong interaction between Acon1_001440 and EGFR, suggesting that Acon_001440 has a high potential to effectively inhibit EGFR activity. The root mean square deviation (RMSD), root mean square fluctuation (RMS), radius gyration (RG), hydrogen bond analysis, and solvent-accessible surface area (SASA) were used to analyze the conformational stability and fluctuation under the simulated condition.

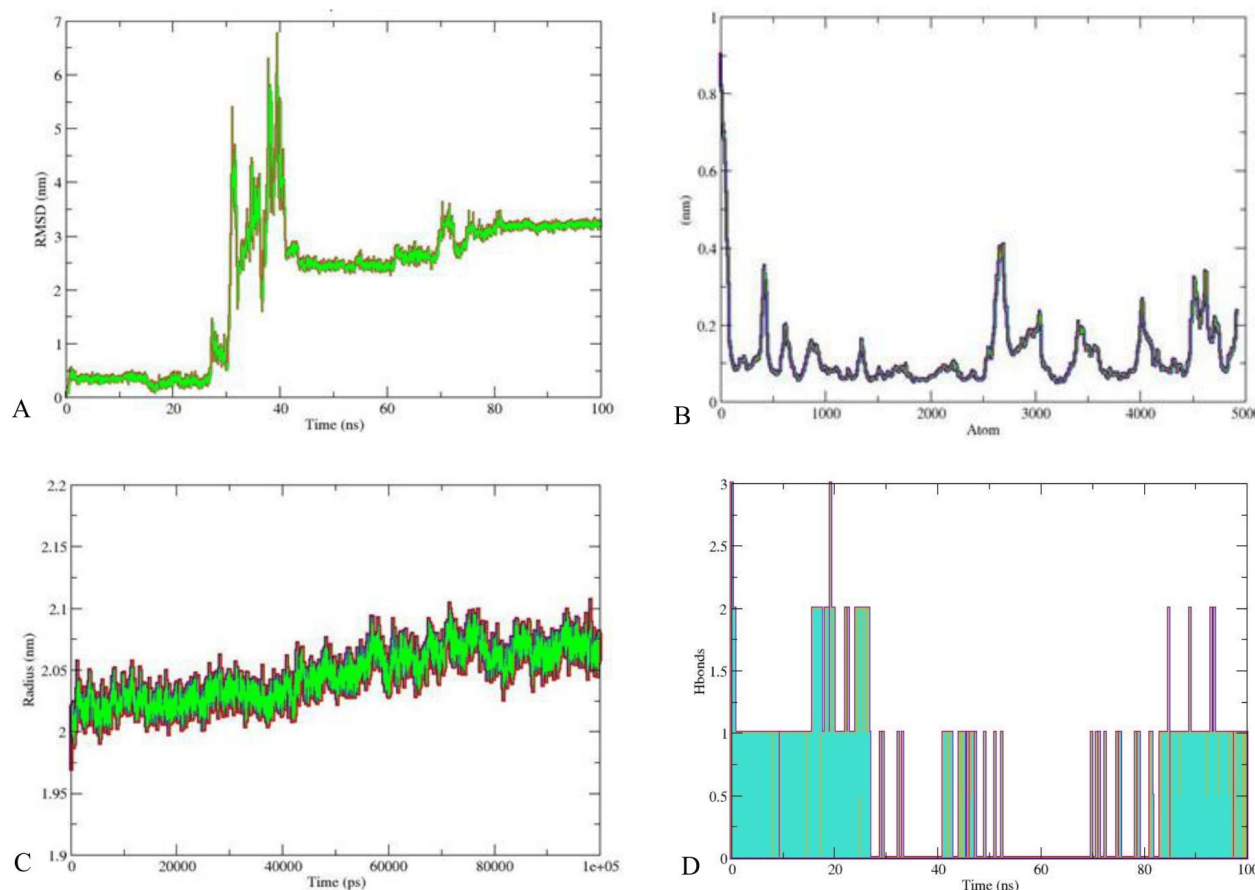


Figure 8. Molecular dynamics simulations performed for the Acon1_001440- EGFR complex. (A) Time evolution of the root means square deviation (RMSD) showing the structural stability of the complex during the simulation. (B). Root means square fluctuation (RMSF) of amino acid residues, indicating local flexibility along the protein structure. (C) Radius of gyration (Rg) as a function of time, reflecting the compactness and overall structural stability of the complex. (D) Number of hydrogen bonds formed between Acon1_001440 and the EGFR throughout the simulation.

The RMSD profile (Figure 8A) describes the structural deviation of the protein-ligand complex relative to the starting conformation during the 100 ns simulation. During the initial phase (0.25 ns), the RMSD values remained relatively low (~0.3 – 0.5 nm), indicating that the complex maintained a conformation close to the starting structure. A notable increase in RMSD occurred between 30 and 40 ns, where values temporarily rose to approximately 6-7 nm, suggesting a substantial conformational rearrangement of the system. Such a transition often corresponds to ligand accommodation within the binding pocket or protein structural adjustment during equilibration. Following this transition, the RMSD stabilized between ~2.5 – 3.3 nm from 40 ns onward, forming a plateau that persisted until the end of the simulation. The convergence of the RMSD trajectory after the mid-simulation phase indicates that the EGFR-Acon1_001440complex reached a dynamically stable conformation, suggesting that the ligand remained accommodated within the binding region throughout the remainder of the simulation.

The RMSF profile (Figure 8B) represents the flexibility of individual residues across the simulation trajectory. Most residues exhibited low fluctuations (<0.2 nm), suggesting a generally stable protein backbone during the simulation. Several peaks were observed, notably around residues indices ~600-700, ~2600-2800, and ~4200-4600, where

fluctuations approached 0.3-0.4 nm. These elevated RMSF values likely correspond to loop regions or surface-exposed segments, which are inherently more flexible than structured secondary elements such as α -helices or sheets. and β - sheets. The absence of large fluctuations (>0.5 nm) across most residues indicates that ligand binding did not induce major destabilization of the EGFR structure, supporting the structural compatibility of Acon_1_001440 with the receptor.

The radius of gyration (Figure 8C) measures the compactness of the protein structure throughout simulation. The Rg values remained relatively stable within the range of ~ 2.00 $\pm$ 0.10 nm across the trajectory. A slight, gradual increase in RG after approximately 40 ns was observed, which corresponds temporally with the structural adjustment detected in the RMSD plot. Despite this minor expansion, the RG values remained within a narrow range, indicating that the overall tertiary structure of EGFR remained compact and structurally stable during ligand binding. This stability suggests that the interaction with Acon1_001440 did not induce significant unfolding or destabilization of the protein structure.

Hydrogen bond analysis (Figure 8D) provides insight into the stability of intermolecular interaction between EGFR and Acon 1_001440. The number of hydrogen bonds fluctuated between 0 to 3 throughout the simulation. Notably, hydrogen bonds were more frequently observed during the early phase (0- 25 ns) and again in the final phase (70- 100 ns) of the simulation. These recurring interactions suggest that hydrogen bonding contributes to stabilizing the ligand within the binding site. Transient hydrogen bonding is common in protein-ligand complexes and may reflect flexible binding modes that allow the ligand to adapt to conformational fluctuations within the receptor pocket.

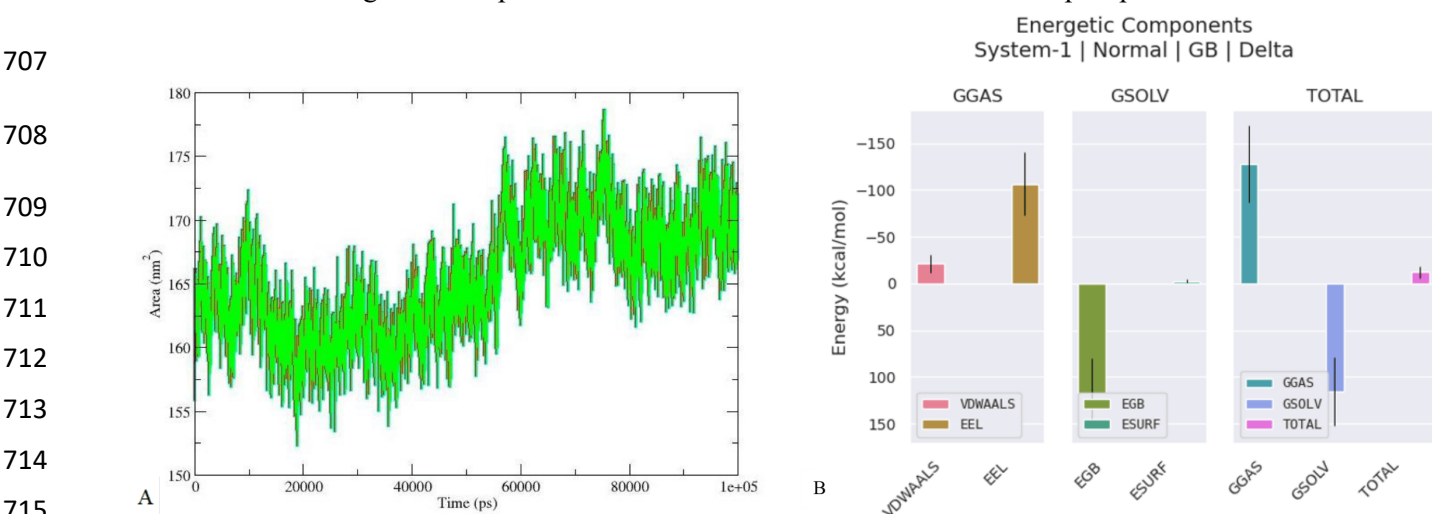


Figure 9. Solvent accessible surface area (SASA) profile and MM/GBSA binding free energy decomposition of the EGFR-Acon1_001440 complex during the 100 ns molecular dynamics simulation.

The solvent-accessible surface area (SASA) of the EGFR-Acon1_001440 complex was monitored during the 100 ns dynamics simulation to evaluate structural stability and solvent exposure. The SASA values fluctuated within a narrow range of approximately 155-178 $\text{\AA}^2$, indicating moderate conformational flexibility while remaining overall structural integrity. An initial decrease from $\sim 165 \text{\AA}^2$ to $\sim 158 \text{\AA}^2$ during the first 0-20 ns suggests slight structural compaction following ligand binding. Between 20 – 50 ns, SASA values remained relatively stable around 160 $\text{\AA}^2$, indicating equilibration of the complex. After 50 ns, a gradual increase was observed, reaching $\sim 175 - 178 \text{\AA}^2$ toward the end of the trajectory, which may reflect minor conformational rearrangement and increased solvent exposure of the surface residues. Despite these fluctuations, no abrupt changes were detected, suggesting that the EGFR-Acon1_001440 complex remained structurally stable throughout the simulation, consistent with the typical dynamic motion observed in stable biomolecular systems. A change in protein surface exposure was assessed.

The binding affinity of the EGFR-Acon_001440 complex using the Molecular Mechanics General Born/Poisson-Boltzmann Surface Area (MM-GB/PBSA) method, yielding a total binding free energy (ΔG_{bind}) of -12.28 kcal/mol, indicating a thermodynamically favorable interaction. The binding process was primarily driven by favorable gas-phase interaction (GGAS = -127.61 kcal/mol), with major contributions from electrostatic interactions (EEL = -106.53 kcal/mol) and van der Waals forces (VDWAALS = -21.07 kcal/mol). These results highlight the important role of electrostatic attraction, hydrogen bonding, and hydrophobic contacts in stabilizing the ligand within the binding pocket. In addition, the nonpolar solvation contribution (ESURF = -2.63 kcal/mol) provided a small stabilizing effect associated with hydrophobic surface burial upon ligand binding. These energetic contributions support the formation of a stable EGFR-Acon1_001440 complex with favorable binding affinity.

3.5. *In silico* cytotoxicity prediction

Table 9 summarizes the *in-silico* cytotoxicity prediction of the selected bioactive compounds across multiple cancer cell lines, tumor types, and tissues. Cytotoxic potential was evaluated using the probability of activity (PA) and probability of inactivity (PI), where higher PA values combined with low PI values indicate a stronger likelihood of anticancer activity. Usnic acid (A) exhibited the strongest predicted cytotoxicity, showing a high PA value of 0.767 and a very low PI value of 0.005 against the U-251 glioma cell line derived from brain tissue. Similarly, Acon1_001440 (E) demonstrated substantial cytotoxic potential against the Hs 683 oligodendroglioma cell line, with a PA of 0.684 and a PI of 0.012. These results suggest that both compounds may possess selective activity against glioma-related cancer models. Feruloyl tyramine (D) showed moderate predicted cytotoxic activity, with a PA of 0.498 and a PI of 0.008 against the MDA-MB-453 breast adenocarcinoma cell line, indicating a lower but still notable likelihood of anticancer activity. Other compounds, including tryptophan (B), pyrenophorol (F), and the 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate (C), displayed intermediate PA values across pancreatic, melanoma, leukemia, and lung cancer cell lines, suggesting variable and cell line-dependent cytotoxic profiles. Overall, the *in silico* cytotoxicity analysis highlights usnic acid and Acon1_001440 as the most promising candidates based on their high activity probabilities and minimal inactivity scores, particularly against brain-derived cancer cell lines. These findings support the molecular docking and molecular dynamics simulations.

Cod e	Bioactive compound	PA	PI	CELL LINE	CANCER TYPE/CELL TYPE	TISSUE	TUMOR TYPE
A	Usnic acid (CID: 5646)	0.767	0.005	U-251	Glioma	Brain	Glioma
B	Tryptophan (CID: 6035)	0.522	0.004	YAPC	Pancreatic carcinoma	Pancreas	Carcinoma
C	8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate (CID: 51136479)	0.556/0.547	0.021/0.030	HL-60; NCI-H838	Promyeloblast leukemia NSCLS (Stage III)	Hematopoietic/ lymphoid; Lung	Leukemia;Carcinoma
D	Feruloyl tyramine (CID: 5280537)	0.498	0.008	MDA-MB-453	Breast adenocarcinoma	Breast	Carcinoma
E	Acon1_001440 (CID: 23902325)	0.684/0.567	0.012/0.015	Hs683; DMS_114	Oligodendroglioma; Lung carcinoma	Brain; Lung	Glioma; Carcinoma
F	Pyrenophorol (CID: 52918385)	0.589	0.007	UACC-62	Melanoma	Skin	melanoma
		0.537	0.024	DMS-114	Lung carcinoma	lung	carcinoma

Table 9. *In silico* cytotoxicity prediction of selected bioactive compounds from *A. adenophora*.

4. DISCUSSION

A. adenophora exhibits a broad spectrum of pharmacological properties attributed to its diverse secondary metabolites, as identified through GC–MS and UHPLC-QTOF-MS analyses. The predominant volatile component, 2,4,6-tri-tert-butyl-nitrobenzene, is an aromatic compound with the largest peak area, has recently been reported to play a potential role in the management of metabolic syndrome (MetS), including hypertension, abdominal obesity, hyperglycemia, hypertriglyceridemia, type 2 *diabetes mellitus*, and cardiovascular diseases²⁰. Aromatic nitro compounds are of particular interest due to their ability to induce cell death and their cytotoxic effects span a broad biological spectrum, including anti-neoplastic, antibiotic, antihypertensive, tranquilizers, and herbicidal activities²¹. They also play a role in cancer chemotherapy as constituents of nitroimidazole and nitracrine derivatives⁴⁵. Although relatively rare in nature, aromatic nitro compounds are widely utilized in pesticide synthesis and exhibit antiparasitic activity⁴⁶.

In addition, several sesquiterpenes were detected in *A. adenophora*, including modephene, silphene, germacrene D, squalene, caryophyllene, and caryophyllene oxide. Modephene demonstrates anticancer, antioxidant, and antibacterial activities as reported in *Pulicaria crispa* essential oil⁴⁷; and also exhibits anti-septic and anti-inflammatory effects in *Atractylodes macrocephala*⁴⁸. Silphene is known for its antifeedant properties⁴⁹, while germacrene D has insecticidal, herbivore-deterrent, and aphid-repellent activities^{50,51}. Squalene, a multifunctional triterpene, has antioxidant, emollient, and drug-carrier roles⁵² and has been associated with antitumor, antimicrobial, anti-inflammatory, and immune-stimulating activities, including anticancer potential against lung, skin, and colon cancers. Caryophyllene has been reported to possess anticonvulsant, anxiolytic, and antidepressant effects⁵³.

Phenolic compounds such as isoferulic acid, ferulic acid, caffeic acid, 2',3',4', Tri(trimethylsilyl)oxyacetophenone were also identified, exhibiting antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. These compounds act through mechanisms such as growth inhibition, apoptosis induction, and cancer risk reduction^{54,55}. Likewise, erythritol and meso-erythritol, classified as sugar alcohols, demonstrated strong antioxidant potential by scavenging hydroxyl radicals and preventing oxidative hemolysis⁵⁶. Furthermore, several sugars and carbohydrates were detected in the ethanolic leaf extract of *A. adenophora*, including D and DL – DL-arabinose, D-fructose, 1,3,4,5,6-pentakis-O(trimethylsilyl)-, O-methyloxime, D- (+)-Turanose, octakis (trimethylsilyl) ether, D-Trehalose, and derivative, as well as silylated carbohydrate derivatives. These biomolecules are essential in cell-to-cell communication and immune responses⁵⁷. Additionally, glyceride-related compounds such as glycerol lupeol trimethylsilyl ether and Stigmast-5-ene, 3 β -(trimethylsiloxy)-(24S)- exhibited anticancer activity through multiple mechanisms, including apoptosis induction, cell cycle arrest, angiogenesis inhibition, and metastasis suppression, often by targeting proteins like Na⁺/K⁺ ATPase,⁵⁸.

Other minor compounds identified include L-valine, an amino acid with reported immunosuppressive effects, and propanoic,3-(2-naphthalenyl)-, an ester-carboxylic acid with colonic barrier-enhancing, insulin-sensitizing, and anti-lipid activities⁵⁹. The non-volatile constituents of *A. adenophora* predominantly comprise phenylpropanoids, flavonoids, alkaloids, fatty acids, terpenoids, and phenolic compounds, which collectively account for most of its pharmacologically active metabolites. Minor components such as aromatic polyketides, peptides, glycopospholipids, and carbohydrates also contributed to its diverse biological activities. Phenylpropanoids, including caffeic acid, 4-methoxycinnamic acid, 3-O-feruloylquinic acid, and cynarin, represent the major identified class and exhibit strong anticancer activity by modulating signal transduction and inducing apoptosis⁶⁰. Caffeic acid, in particular, demonstrates potent anti-inflammatory and anti-cancer effects⁶¹. Detected flavonoids such as quercetin, hyperoside, 4-methoxydalbergione have been associated with apoptosis induction, inhibition of Akt, mTOR/ ERK pathways, and suppression of angiogenesis^{62,63}. Alkaloids, widely known for their antiproliferative and cytotoxic effects, further enhance the plant's therapeutic potential, with isoquinoline derivatives showing anticancer, antimicrobial, and anti-inflammatory activities^{64,65}. Additionally, fatty acids identified in the extract are linked to apoptosis induction and inhibition of cell migration, invasion, and angiogenesis^{44,58 44,66,67}, anticancer properties, inhibits CHO-K1 and melanoma cell lines⁶⁸, 6-hydroxypalmitic acid is a candidate for anticancer drug development⁶⁹, and targets breast cancer stem cells by downregulating c-Myc⁵⁸. The diverse secondary metabolites of *A. adenophora* highlight its broad pharmacological potential and reinforce its value as a promising source of bioactive compounds for anticancer drug development.

In early-stage drug discovery, systematic evaluation of physicochemical properties, pharmacokinetics, drug-likeness, and medicinal chemistry attributes is essential for prioritizing viable therapeutic candidates. Such assessments enable the prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles, thereby reducing the likelihood of failure during later stages of drug development. Importantly, a compound must demonstrate not only biological activity but also favorable pharmacokinetic behavior, adequate bioavailability, and acceptable safety characteristics to be considered a credible drug candidate⁷⁰.

Lipophilicity is a key physicochemical determinant influencing drug–target interactions, passive diffusion across biological membranes, and solubility within lipid environments. These properties collectively shape both pharmacokinetic and pharmacodynamic performance and are central to ADMET behavior⁶⁴. An optimal lipophilicity range, typically between 0 and 5, is widely regarded as favorable for balancing aqueous solubility with membrane permeability^{71,72}. In the present study, all six evaluated compounds exhibited lipophilicity values within this optimal range, suggesting efficient membrane transport and potential for favorable systemic exposure.

Solubility is another critical parameter governing drug absorption and bioavailability, as it determines the extent to which a compound can dissolve in biological fluids, particularly within the gastrointestinal tract⁷³. Adequate aqueous solubility at the site of absorption is required for effective uptake and subsequent systemic distribution⁷⁰. All six compounds displayed moderate solubility, indicating favorable dissolution characteristics and supporting their potential suitability for oral administration.

ADME profiling further characterizes absorption, distribution, metabolism, and excretion properties that determine systemic exposure and therapeutic efficacy⁷⁴. Efficient gastrointestinal absorption is a fundamental requirement for orally administered drugs, as it directly impacts systemic availability. All six compounds evaluated in this study demonstrated favorable predicted GI absorption, supporting their potential as orally bioavailable candidates. Notably, Acon1_001440 also showed predicted central nervous system (CNS) permeability, suggesting possible neuropharmacological relevance. Together, these findings highlight the overall favorable pharmacokinetic profiles of the evaluated compounds and provide a rational basis for further optimization and experimental validation in subsequent drug development studies.

P-glycoprotein (P-gp) is a glycosylated membrane-bound efflux transporter that plays a crucial role in modulating drug disposition by actively extruding xenobiotics and therapeutic agents from cells, thereby reducing their intercellular and systemic concentration⁷⁵. In this study, feruloyl tyramine was predicted to be a non-substrate of P-gp, while remaining compounds may undergo efflux-mediated transport, potentially decreasing their intracellular accumulation and consequently may undergo efflux-mediated transport. Such active extrusion could decrease intracellular accumulation, potentially lowering their therapeutic efficacy due to reduced bioavailability at the target site. Furthermore, cytochrome P450 (CYP) isoenzymes are key mediators of oxidative metabolism and xenobiotic clearance. Inhibition of these enzymes can impair drug elimination, alter pharmacokinetic behavior, and increase the risk of toxicity⁷⁶. The current analysis revealed that usnic acid, tryptophan, 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate, Feruloyltyramine, along with the reference drug eprenatapopt, exhibited low inhibitory potential toward major CYP isoforms, thereby minimizing the likelihood of drug accumulation, toxicity, and adverse pharmacokinetic interactions.

P-glycoprotein (P-gp) is a glycosylated, membrane-bound efflux transporter that plays a crucial role in modulating drug disposition by actively extruding xenobiotics and therapeutic agents from cells, thereby reducing their intracellular and systemic concentrations⁷⁶. In this study, feruloyl tyramine was predicted to be a non-substrate of P-gp, whereas the remaining compounds were predicted to undergo efflux-mediated transport, which may reduce intracellular accumulation and consequently limit bioavailability at the target site. Such active extrusion could diminish therapeutic efficacy by lowering effective intracellular drug concentrations.

Cytochrome P450 (CYP) isoenzymes are key mediators of oxidative metabolism and xenobiotic clearance. Inhibition of these enzymes can impair drug elimination, alter pharmacokinetic behavior, and increase the risk of toxicity⁶⁷. The current analysis revealed that usnic acid, tryptophan, 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate, feruloyl tyramine, along with the reference drug eprenatapopt, exhibited low inhibitory potential toward major CYP isoforms. This suggests a reduced likelihood of metabolic interference, drug accumulation, and adverse pharmacokinetic interactions.

Drug-likeness evaluation provides an important predictive framework for assessing the feasibility of compounds as drug candidates by comparing their physicochemical and structural properties with those of approved therapeutic agents. This approach facilitates early exclusion of molecules with unfavorable pharmacokinetic or bioavailability profiles, thereby reducing development costs and minimizing late-stage failures⁷⁷. In this study, five established computational filters - Lipinski, Ghose, Veber, Egan, and Muegge - were applied to predict the oral drug-likeness of the selected compounds. These models collectively assess molecular weight, lipophilicity (log P), hydrogen bond donors and acceptors, topological polar surface area, and rotatable bonds, which are key determinants of absorption and permeability. All six compounds satisfied the thresholds across these filters, indicating favorable drug-like characteristics and supporting their potential as promising lead candidates for further pharmacokinetic and preclinical evaluation.

The selected bioactive compounds were further screened for pan-assay interference compounds (PAINS) and BRENK alerts to minimize the likelihood of false-positive hits arising from promiscuous or reactive chemical motifs. Notably, Acon1_001440 exhibited no PAINS or BRENK alerts, indicating a chemically clean scaffold and supporting the reliability of its predicted bioactivity during *in silico* screening⁷⁰.

In silico toxicity profiling using ProTox-II provided an initial assessment of the safety profiles of the selected compounds by evaluating acute toxicity (LD₅₀), organ toxicity, genotoxicity, and carcinogenicity. Both Acon1_001440 and pyrenophorol exhibited LD₅₀ values exceeding 2000 mg/kg, suggesting low acute oral toxicity. No organ-specific toxicities were predicted for these compounds; however, Acon1_001440 was flagged for potential carcinogenicity, warranting cautious interpretation and experimental validation.

The drug-likeness and pharmacokinetic suitability of the compounds were further evaluated using the bioavailability radar model, which integrates six key physicochemical descriptors such as lipophilicity, size, polarity, solubility, flexibility, and saturation, to provide a visual assessment of oral drug-likeness. Usnic acid, 8,8-dimethyl-2,10-dioxo-9H-pyrano[2,3-f]chromen-9-yl (Z)-2-methylbut-2-enoate, 7-(2E,5E)-7-hydroxy-3,7-dimethylocta-2,5-dienoxychromen-2-one, Acon1_001440, and pyrenophorol were entirely enclosed within the optimal region of the radar

plot, indicating favorable oral bioavailability profiles. In contrast, tryptophan and feruloyl tyramine deviated from the acceptable saturation range, suggesting potential limitations related to membrane permeability or metabolic stability. Compared with the reference anticancer agents eprenetapopt (APR-246) and erlotinib, which partially fell outside the optimal region, several *A. adenophora* derived compounds demonstrated comparable or superior drug-likeness⁷⁸. These findings suggest that the identified phytochemicals possess physicochemical properties conducive to efficient systemic absorption and distribution, supporting their potential as drug candidates⁷⁹.

Among the evaluated compounds, Acon1_001440 consistently displayed the most favorable ADMET and drug-likeness profile, justifying its selection for detailed molecular docking and simulation analyses. Molecular docking remains a cornerstone of structure-based drug discovery, enabling the prediction of ligand-protein binding modes and affinities, with lower binding energies indicative of stronger interactions⁸⁰. Docking simulations were performed against four cancer-related targets with established roles in oncogenesis: EGFR, p53, MMP7, and CDK8/Cyclin C. EGFR regulates key signaling pathways involved in cell proliferation and survival⁸¹, p53 protein maintains genomic integrity and suppresses malignant transformation⁸², MMP7 promotes tumor invasion and angiogenesis through extracellular matrix degradation⁸³, CDK8 modulates transcriptional control and cell-cycle progression⁸⁴. Collectively, these proteins represent mechanistically diverse but clinically relevant anticancer targets.

Acon1_001440 exhibited strong binding affinities toward all four targets, forming stable ligand-protein complexes through multiple non-covalent interactions. Within the EGFR binding pocket, the compound established conventional hydrogen bonds alongside π -alkyl and van der Waals interactions, facilitating effective accommodation within the active site. Interaction with p53 was dominated by hydrophobic contacts and van der Waals forces, suggesting favorable surface complementarity. In the MMP7 complex, van der Waals interactions, π -alkyl, and carbon-hydrogen bonds contributed to complete stabilization. Although Acon1_001440 did not directly engage the catalytic residues of CDK8/Cyclin C, it formed hydrogen bonds and van der Waals interactions with residues proximal to the active site cleft, which may induce local conformational perturbations capable of modulating enzymatic activity⁸⁵. Overall, hydrogen bonding and van der Waals forces emerged as the dominant contributors to binding stability, supported by hydrophobic interactions, π -alkyl, and π - π stacking interactions⁸⁶. These interaction patterns are consistent with stable and selective ligand binding, supporting the hypothesis that Acon1_001440 may function as a multi-target anticancer modulator.

The predicted biological activity of Acon1_001440 was further explored using CLC-Pred, based on the PASS algorithm, which reports an average predictive accuracy of approximately 96% when benchmarked against experimental *in vivo* data. The analysis indicated potential cytotoxic activity against Hs 683 oligodendroglioma cells and lung carcinoma models, providing complementary evidence for its anticancer relevance. While PASS-based predictions cannot replace experimental validation, they offer valuable hypothesis-generating insights that align with the docking and ADMET findings.

To further assess the dynamic stability of ligand-protein interaction, molecular dynamics (MD) simulations were conducted using the EGFR-Acon1_001440 complex as a representative system⁸⁷. The RMSD of the protein backbone initially showed minor deviations from the starting structure, followed by stabilization after ~40ns, indicating that the complex reached a conformationally equilibrated state with the ligand remaining bound. Such RMSD stabilization has been widely used as an indicator of complex stability in EGFR inhibitor studies. Isa et al. (2025) reported minimal RMSD fluctuations over 100ns for stable EGFR-ligand complexes in a similar MD framework⁸⁸. The root mean square fluctuation (RMSF) profile revealed low flexibility (<0.2 nm) across most residues, with slightly elevated motion in loop regions typical of surface flexibility rather than destabilization of the binding domain.

Analysis of Rg showed consistent values throughout the trajectory, indicating that the overall protein compactness was maintained, a pattern also observed in the EGFR MD studies, where ligand binding did not perturb global structure⁸⁸. Hydrogen bond analysis demonstrated intermittent hydrogen bonding between Acon1_001440 and EGFR throughout the simulation, suggesting that dynamic polar interactions contribute to binding stability, consistent with previously reported EGFR MD results. The SASA remained within a narrow range (155 – 178 Å²) without abrupt changes, indicating that the complex maintained structural integrity while undergoing normal conformational fluctuations.

MM/GBSA binding free energy calculations yielded a favorable ΔG_{bind} of -12.28 kcal/mol, driven by significant van der Waals and electrostatic contributions, reinforcing that both hydrophobic contacts and polar interactions are key stabilizing forces. Such energy component patterns are in agreement with other computational analyses of EGFR inhibitors, where favorable non-bonded interactions outweigh solvation penalties to produce stable complexes⁸⁸. These results suggest that Acon1_001440 forms a stable and energetically favorable complex with EGFR, supporting its potential as a computationally predicted inhibitor candidate.

Despite these promising in silico findings, several limitations must be acknowledged. This study relies primarily on computational predictions, which, although powerful for early-stage screening, cannot fully capture the complexity of biological systems. Critical factors such as metabolic transformations, protein expression variability, immune interactions, and interindividual differences were not addressed. Furthermore, toxicity and cytotoxicity predictions, including those suggesting carcinogenic potential, are model-dependent and require experimental confirmation. Although erlotinib and eprentapopt (APR-246) were included as reference compounds, no direct experimental comparisons were performed, limiting conclusions regarding relative clinical efficacy or synergistic potential.

Additionally, only four cancer-related targets were evaluated, and EGFR was selected for detailed molecular dynamics analysis due to its central role in multiple cancer types. Expanding future analyses to include additional signaling pathways and off-target proteins would provide a more comprehensive assessment of therapeutic scope and safety. Practical considerations relevant to drug development, such as chemical stability, synthetic feasibility, scalability, and formulation, were beyond the scope of this study and remain to be investigated.

Overall, Acon1_001440 emerged as the most promising *A. adenophora*-derived compound, demonstrating favorable drug-likeness, acceptable predicted toxicity, robust multi-target binding, and dynamic stability in silico. While these findings position Acon1_001440 as a compelling lead candidate, systematic in vitro and in vivo validation is essential to confirm its anticancer efficacy, pharmacokinetic behavior, and safety profile prior to translational development.

5. CONCLUSION

Ageratina adenophora contains a diverse array of bioactive secondary metabolites, including aromatic nitro compounds, sesquiterpenes, phenolics, flavonoids, alkaloids, and glyceride derivatives, many of which have been reported to exhibit anticancer activities. Among these, Acon1_001440 displayed the most favorable combination of predicted drug-likeness, pharmacokinetic properties, and oral bioavailability. In silico docking and molecular dynamics simulations suggested stable, multi-target interactions with EGFR, p53, MMP7, and CDK8/Cyclin C, supported by favorable binding free energies and conformational stability, indicating its potential as a multi-target anticancer candidate.

While these findings are promising, they are based on computational predictions and require systematic in vitro and in vivo validation to confirm efficacy, safety, and pharmacologically active compounds. Thus, Acon1_001440 may be considered a promising lead candidate for further investigation in anticancer drug development.

Data availability

The data presented in this study are available in this article. Further inquiries can be directed to the corresponding authors.

Author contributions

E.E.P.: Sample collection, Methodology, Data curation, Formal analysis, Resources, Software, Visualization, Writing – original draft; K.G.J.C.: Conceptualization, Supervision, Validation, Data curation, Formal analysis, Investigation, Methodology, Writing & Reviewing; A.K.C.L.: Methodology, Formal analysis; A.B.M.M.K.I.: Methodology, Formal analysis; K.W.: Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Competing interests

The authors declare no competing interests.

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