Betulinic Acid and Taccalin as Potential Modulators of Granulysin-Mediated Keratinocyte Apoptosis in Erythema Multiforme

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Abstract

Background: Erythema multiforme (EM) is an immune-mediated mucocutaneous disorder characterized by keratinocyte apoptosis, with granulysin (GNLY) identified as a key cytotoxic mediator. Targeting GNLY may offer a promising strategy for developing more specific and safer therapeutic interventions.
Objective: To evaluate the potential of phytochemicals derived from *Tacca integrifolia* as modulators of GNLY-mediated apoptosis using computational approaches.
Methods: Protein–protein interaction (PPI) analysis of GNLY was performed using the STRING database to identify its role in apoptotic and immune pathways. Selected phytochemicals—taccalin, betulinic acid, gamma-aminobutyric acid (GABA), and guaijaverin—were retrieved and assessed for their interaction with GNLY. Drug-likeness and pharmacokinetic properties were evaluated using standard ADMET prediction tools.
Results: PPI analysis revealed GNLY as a central hub interacting with key cytotoxic proteins, including perforin and granzymes, highlighting its role in immune-mediated apoptosis. Interaction analysis demonstrated favorable binding affinities ranging from –3.4 to –6.8 kcal/mol, with betulinic acid and taccalin showing the strongest interactions. Drug-likeness and ADMET evaluation identified taccalin, betulinic acid, and GABA as promising candidates with favorable pharmacokinetic profiles, including non-permeability to the blood–brain barrier and low predicted toxicity.
Conclusion: Phytochemicals derived from *Tacca integrifolia*, particularly taccalin, betulinic acid, and GABA, show potential as modulators of GNLY-mediated apoptosis. These findings highlight a novel GNLY-targeted therapeutic approach for erythema multiforme and warrant further validation through molecular dynamics simulations and experimental studies.

Keywords: Green products, skin, disease, health, genetics

Introduction

Erythema multiforme (EM) is an acute, immune-mediated mucocutaneous disorder characterized by target-like skin lesions and, in severe cases, mucosal surfaces including ocular, oral, and genital mucosa, may also be involved (Hafsi et al, 2026). Although its exact pathogenesis is complex, cytotoxic T lymphocytes and natural killer (NK) cells are known to play central roles in epithelial cell damage (Kechichian E et al, 2024). Among the effector molecules released by these immune cells, Granulysin (GNLY) has emerged as a critical mediator of keratinocyte apoptosis, thereby driving tissue injury in EM (Chen CB et al, 2020). Elevated GNLY expression has been consistently associated with disease activity, making it a promising molecular target for therapeutic intervention (Huyen TT et al, 2026).

GNLY is a cytolytic protein secreted by activated cytotoxic T lymphocytes and natural killer cells. Beyond its antimicrobial activity, GNLY has been recognized as a major mediator of keratinocyte apoptosis through membrane disruption and activation of apoptotic cascades (Drvar V et al, 2022). Elevated levels of GNLY have been detected in blister fluid and serum of patients with severe cutaneous adverse reactions, correlating with disease severity (Stewart TJ et al, 2024). Therefore, inhibition of GNLY-mediated cytotoxicity may represent a targeted therapeutic strategy capable of reducing epithelial damage while preserving broader immune functions.

Current management strategies for EM are largely centered on symptomatic relief through the use of corticosteroids, immunosuppressive agents such as azathioprine and mycophenolate mofetil, monoclonal antibodies like rituximab, and antiviral drugs. However, these treatments often offer only partial improvement and may be associated with significant adverse effects (Cai Y et al, 2024). In contrast, the therapeutic landscape for pemphigus is advancing rapidly, with the emergence of several novel treatment approaches, including neonatal Fc receptor (FcRn)-targeting agents such as efgartigimod and ALXN1830, Bruton's tyrosine kinase (BTK) inhibitors, chimeric antigen receptor (CAR) T-cell therapy, as well as intralesional and combination therapies (Lim YL et al, 2022). Despite growing insights into the immunopathology of EM, no specific GNLY-targeted inhibitors are currently available in clinical practice. This has led to increasing interest in the investigation of natural compounds as potential alternative or adjunctive therapies, particularly through in silico approaches that enable rapid screening of bioactive molecules with possible inhibitory activity against GNLY.

Tacca integrifolia, commonly known as the white batflower or black lily, is a medicinal plant traditionally used in Southeast Asia for the treatment of fever, inflammation, and skin disorders (Shwe HH et al, 2010). Previous studies suggest that taccalonolides derived from T. integrifolia act as microtubule stabilizers and may serve as potential therapeutic agents for neurodegenerative disorders, including Alzheimer's disease and tauopathies (Peng

J et al, 2011). Phytochemical investigations of *Knautia integrifolia* & *Holoptelea integrifolia* have revealed the presence of bioactive compounds such as quinic acid, chlorogenic acid, isoquercitrin, hesperidin, genistin, and unique triterpenoids, many of which exhibit antioxidant, anti-inflammatory, and anticancer properties (Kılınc H 2025; Mohammed HS et al, 2023). Despite its pharmacological potential in inhibiting the cancer growth & induce cell death, *T. integrifolia* has not yet been systematically explored for its role in modulating GNLY-mediated apoptotic pathways, leaving its therapeutic relevance in EM largely unexplored (Ahmed S et al, 2019).
Several phytoconstituents of *Tacca integrifolia* possess anti-inflammatory, antioxidant, and immunomodulatory properties (Chatsakulphanit C et al, 2026; Armartmuntree N et al, 2025). These activities suggest a potential role in modulating cytotoxic immune pathways associated with keratinocyte injury. However, their interaction with GNLY has not been investigated previously. In light of these gaps, the present study employed an *in-silico* approach to evaluate the inhibitory potential of phytochemicals from *T. integrifolia* against GNLY. To the best of our knowledge, this is the first report integrating protein–protein interaction analysis, molecular docking, and ADMET profiling of *T. integrifolia* phytochemicals targeting GNLY in erythema multiforme. By identifying drug-like compounds with favorable binding affinities and pharmacokinetic properties, this work introduces a novel therapeutic strategy for managing EM.

Methodology

Retrieval of Target Protein

The amino acid sequence of Granulysin (GNLY), a cytolytic protein implicated in apoptosis and immune-mediated tissue injury, was retrieved from the UniProt database (P22749 · GNLY_HUMAN) in format (https://www.uniprot.org/uniprotkb/P22749/entry) (Sami MRS et al, 2024). For docking purposes, the three-dimensional (3D) structure of GNLY was obtained from the Protein Data Bank (https://www.rcsb.org/structure/1L9L). In cases where a crystallographic structure was unavailable, homology modeling was performed using SWISS-MODEL, and the model was validated by Ramachandran plot analysis and ProSA-web to ensure structural reliability (Berman HM et al, 2000).

Protein–Protein Interaction (PPI) Analysis

The STRING database (version 12.0) was employed to investigate the protein–protein interaction network of GNLY (https://string-db.org/). Parameters were set with a high confidence score (0.7), and Homo sapiens was selected as the organism (Szklarczyk D et al, 2023; Rao Vs et al, 2014). The generated PPI network was visualized to identify key interacting partners and functional associations, highlighting GNLY's role in immune and apoptotic pathways.

Retrieval of Phytochemicals from *Tacca integrifolia*

Phytochemical constituents of *Tacca integrifolia* were retrieved from the IMPPAT (Indian Medicinal Plants, Phytochemistry, and Therapeutics) database (Mohanraj K et al 2018). Reported bioactive compounds were shortlisted, and their 2D structures were downloaded in SDF format. The ligands were then converted into PDBQT format after energy minimization using Open Babel.

Molecular Docking Studies

Docking simulations between GNLY and the phytochemicals were conducted using AutoDock Vina (version 1.2.3) (Ramadoss R & Padmanabhan R, 2025). Prior to docking, the protein was prepared by removing water molecules and adding polar hydrogens using AutoDock Tools. A grid box was generated to cover the predicted active/binding site of GNLY. Each ligand was docked separately, and the best docking pose was selected based on binding affinity (kcal/mol) and orientation within the active site pocket. Interactions, including hydrogen bonding, hydrophobic contacts, and van der Waals forces, were analyzed using PyMOL and Discovery Studio Visualizer.

ADMET and Drug-Likeness Analysis

The pharmacokinetic and toxicity profiles of docked phytochemicals were evaluated using the SwissADME (https://www.swissadme.ch/) and pkCSM web servers (Daina A et al, 2017). Key parameters assessed included Lipinski's Rule of Five, absorption, distribution, metabolism, excretion, and toxicity (ADMET). Blood–brain barrier (BBB) permeability was also analyzed to assess systemic safety and drug-likeness. Compounds satisfying drug-likeness rules and exhibiting low predicted toxicity were considered as potential leads.

Selection of Potential Lead Compounds

To identify the most promising inhibitors of GNLY, the docked phytochemicals were subjected to a systematic selection process. The primary criterion was docking affinity, where compounds with binding energies ≤ –6.0 kcal/mol were considered to exhibit strong and stable interactions with the target protein. In the next step, drug-likeness was evaluated based on Lipinski's Rule of Five and bioavailability scores, ensuring the shortlisted compounds possessed favorable physicochemical properties for oral administration (Karami TK et al, 2022). Finally, ADMET profiling was performed using computational prediction tools, with special emphasis on blood–brain barrier (BBB) non-permeability to minimize central nervous system penetration, and low predicted toxicity to ensure systemic safety. Compounds fulfilling all three criteria were classified as potential lead candidates for inhibiting GNLY-mediated apoptosis.

Results

Protein–Protein Interaction Analysis of Granulysin

STRING database analysis of granulysin (GNLY) revealed a densely interconnected protein–protein interaction network comprising 11 nodes, highlighting its extensive involvement in immune regulation and apoptotic signaling pathways (Figure 1). GNLY demonstrated strong associations with key cytotoxic effector proteins, including perforin-1 (PRF1), granzymes (GZMA and GZMB), and immune regulatory molecules such as NKG7, KLRD1, FASLG, and CD8A. The high degree of connectivity among these proteins highlighting a coordinated cytotoxic mechanism, reinforcing the central role of GNLY in mediating keratinocyte apoptosis and immune-driven epithelial injury. These findings support the pathological significance of GNLY in erythema multiforme and validate it as a potential therapeutic target.

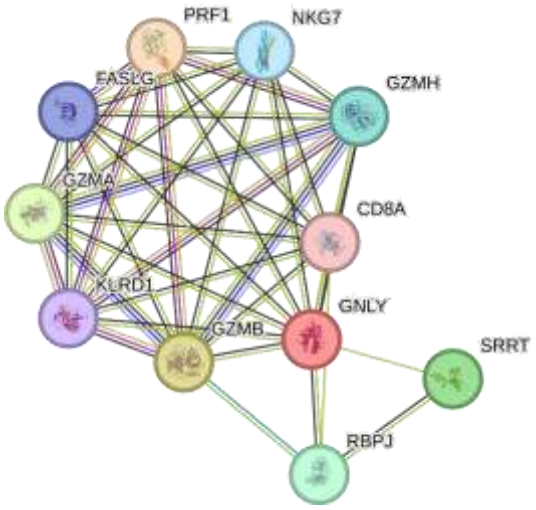

Figure 1. Protein–Protein Interaction Network of GNLY

STRING-derived PPI network showing interactions of GNLY with key cytotoxic and immune-related proteins. The network highlights its central role in apoptosis and immune signaling pathways.

Identification of Phytochemicals from *Tacca integrifolia*

Four phytochemicals—Taccalin, Betulinic acid, Gamma-Amino Butyric Acid (GABA), and Guaijaverin—were retrieved from the IMPPAT database based on their reported pharmacological relevance and structural diversity (Figure 2). These compounds were selected as candidate bioactive molecules for evaluating their interaction potential with GNLY.

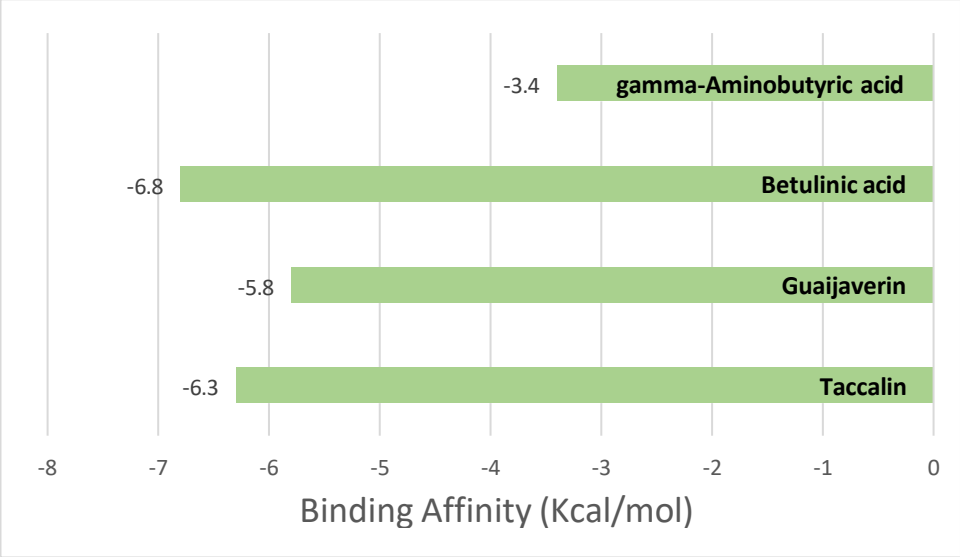

Figure 2. Binding Affinity of Phytocompounds

Graph showing binding affinity values (kcal/mol) of compounds against GNLY. Betulinic acid exhibited the strongest interaction among the selected molecules.

Interaction analysis with GNLY

All four phytochemicals demonstrated favorable interaction profiles with GNLY, with binding affinities ranging from –3.4 to –6.8 kcal/mol (Table 1). Among them, Betulinic acid exhibited the strongest interaction (–6.8 kcal/mol), followed by Taccalin (–6.3 kcal/mol) and Guaijaverin (–5.8 kcal/mol), whereas GABA showed comparatively lower affinity (–3.4 kcal/mol).

Table 1. Interaction of Phytocompounds with GNLY

S.No	Target	Binding Affinity (Kcal/mol)	Interacting amino acids
1	Taccalin	-6.3	Arg50, Arg35, Cys34, Arg46, Met49, Ala30, Thr31, Ser27, Ile57, Gln53
2	Guaijaverin	-5.8	Arg38, Gly37, Arg40, Trp41, Arg2, Arg5, Thr6, Val44, Lys13, Gln12, Thr9
3	Betulinic acid	-6.8	Arg35, Arg46, Thr31, Cys34, Val33, Arg32, Ala30, Met49, Arg50, Gln53
4	gamma-Aminobutyric acid	-3.4	Arg55, Val56, Phe48, Tyr52, Cys7, Ile69, Leu73, Glu71, Asp72, Cys70

Binding poses illustrating interactions of phytocompounds within the GNLY active site. Key amino acid residues involved in binding are highlighted.

Detailed interaction analysis revealed that these compounds engaged critical amino acid residues within the functional regions of GNLY. Notably, Betulinic acid and Taccalin interacted with key residues such as Arg35, Arg46, Cys34, Thr31, Ala30, and Met49, suggesting stable binding within the active region of the protein (Figure 3). Guaijaverin formed interactions with residues including Arg38, Gly37, Trp41, and Lys13, while GABA showed interactions with residues such as Arg55, Val56, and Tyr52, indicating comparatively weaker binding stability. These interaction patterns suggest the potential of selected phytochemicals to modulate GNLY-mediated apoptotic signaling.

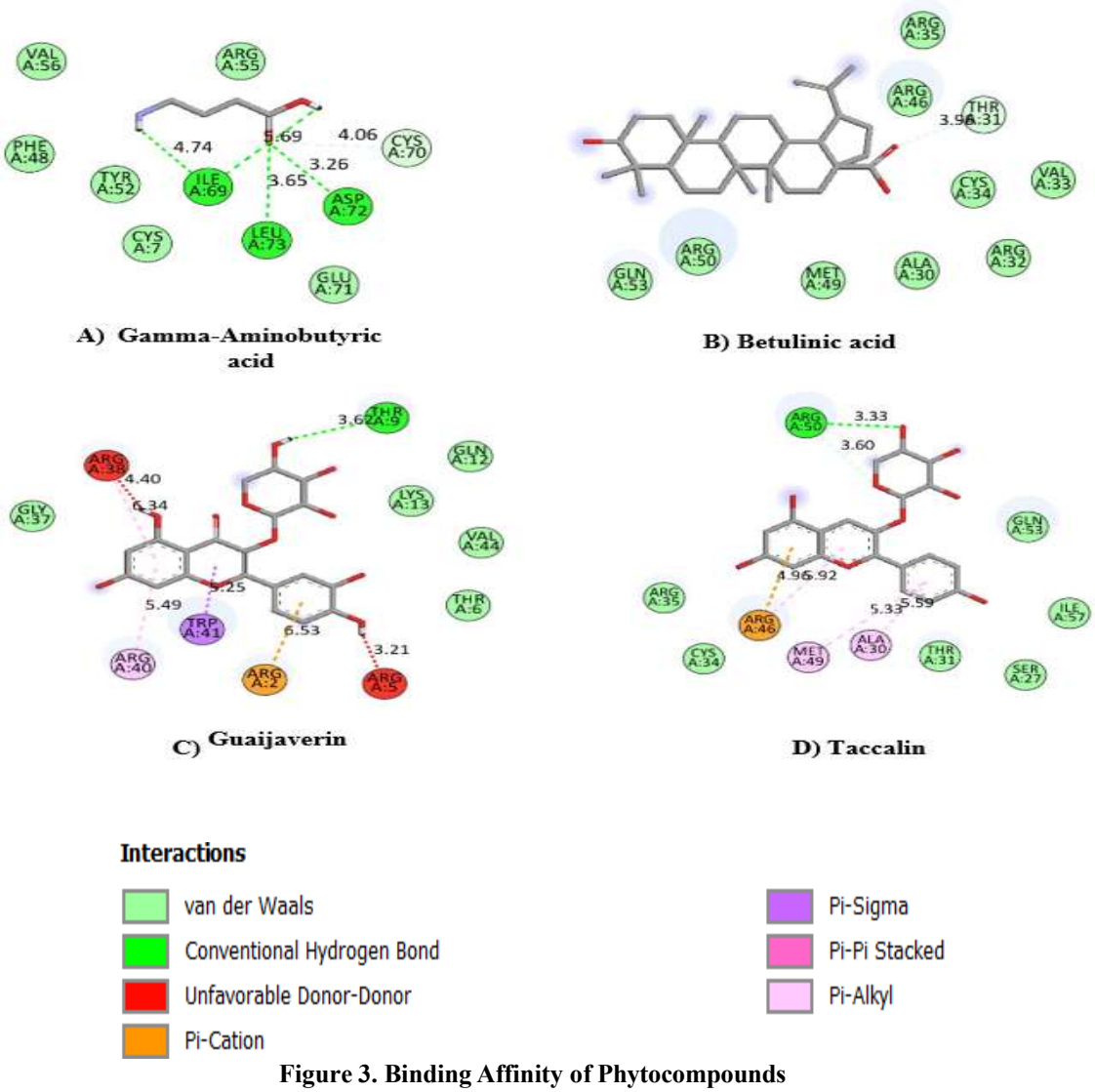

Figure 3. Binding Affinity of Phytocompounds

Binding affinity values (kcal/mol) of compounds (A - GABA, B – Betulinic acid, C – Guaijavrein, D - Taccalin) against GNLY. Betulinic acid exhibited the strongest interaction among the selected molecules.

Drug-Likeness and Pharmacokinetic Properties

Drug-likeness evaluation demonstrated that Taccalin, Betulinic acid, and GABA satisfied key physicochemical criteria, including Lipinski's Rule of Five, indicating favorable oral bioavailability (Figure 4). Pharmacokinetic predictions further revealed that these compounds are non-permeant to the blood–brain barrier (BBB), suggesting reduced likelihood of central nervous system-related adverse effects and supporting their suitability for systemic therapeutic applications.

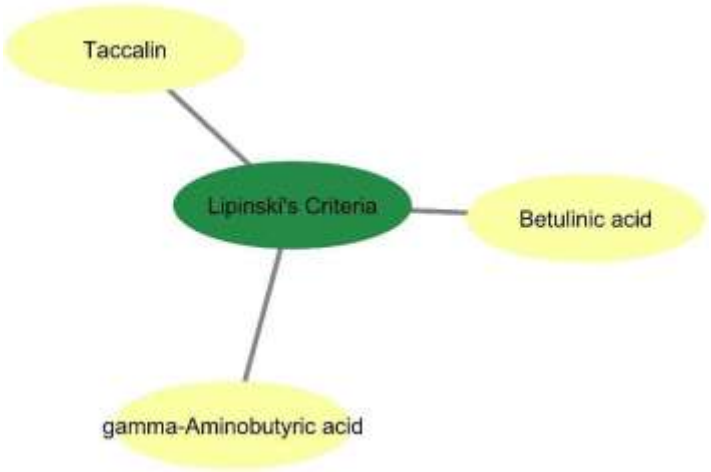

Figure 4. Drug-Likeness Analysis

Summary of drug-likeness evaluation based on standard criteria. Most compounds satisfy key parameters for oral drug potential.

Pharmacokinetic Radar Profiling

The bioavailability radar analysis of the selected phytocompounds revealed distinct physicochemical profiles influencing their drug-likeness and pharmacokinetic suitability (Figure 5). Taccalin exhibited moderate lipophilicity and polarity, with acceptable size and flexibility, although a slight deviation in solubility parameters suggests potential limitations for aqueous dispersion. Betulinic acid demonstrated high lipophilicity and reduced polarity, along with increased insolubility, indicating strong membrane affinity but potentially limited bioavailability. In contrast, GABA displayed favorable polarity and solubility with low lipophilicity and molecular size, aligning well within the optimal drug-likeness space and suggesting efficient absorption characteristics. Overall, the radar plots indicate that while Betulinic acid may favor membrane interactions and stability, Taccalin and GABA present a more balanced pharmacokinetic profile, supporting their suitability as potential therapeutic candidates.

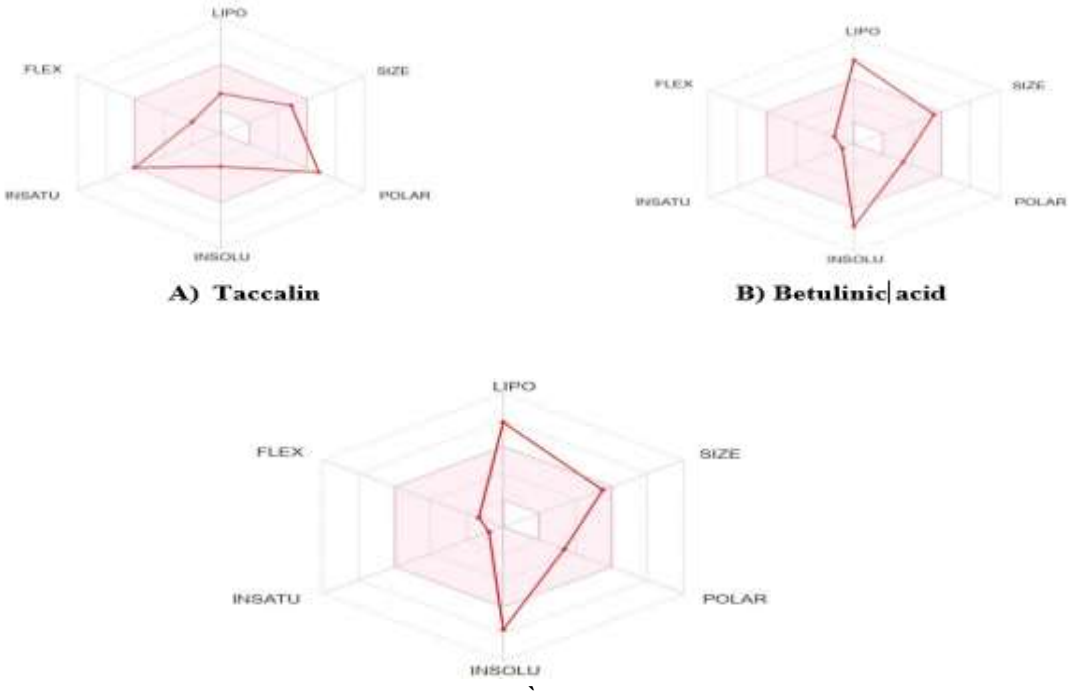

Figure 5. Bioavailability Radar Plot of Selected Phytocompounds

(A) Taccalin, (B) Betulinic acid, and (C) Gamma-Amino Butyric Acid (GABA) showing physicochemical properties relevant to oral bioavailability. The shaded region represents the optimal range, with GABA and Taccalin showing better compliance than Betulinic acid.

Lead Compound Selection

Based on the integrated assessment of binding affinity, interaction profile, drug-likeness, and pharmacokinetic properties, Betulinic acid, Taccalin, and GABA were identified as the most promising lead compounds. Among these, Betulinic acid emerged as the top candidate due to its superior binding affinity and stable interaction with key functional residues of GNLY.

Overall, the findings indicate that phytochemicals derived from *Tacca integrifolia* possess significant potential to modulate granulysin-mediated apoptosis, highlighting their relevance as prospective therapeutic agents for the management of erythema multiforme.

Discussion

The present study delineates a granulysin (GNLY)-centered molecular framework underlying immune-mediated keratinocyte apoptosis and identifies *T. integrifolia*-derived phytochemicals as potential modulators of this pathway. Protein–protein interaction analysis positioned GNLY as a critical hub within a tightly interconnected cytotoxic network involving perforin (PRF1), granzymes (GZMA and GZMB), and immune regulatory molecules such as NKG7, KLRD1, FASLG, and CD8A. This network architecture reflects a coordinated cytotoxic program, reinforcing the concept that GNLY functions upstream in orchestrating epithelial cell death. These findings are highly consistent with established evidence from immune-mediated mucocutaneous disorders, particularly within the Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) spectrum, where GNLY released by cytotoxic T lymphocytes and natural killer cells has been identified as a major mediator of widespread keratinocyte apoptosis (Justice J et al, 2025). Although erythema multiforme (EM) represents a clinically distinct and often less severe condition, the overlap in effector pathways supports the biological plausibility of a GNLY-driven apoptotic axis in its pathogenesis.

Our STRING analysis positioned GNLY at the hub of immune–apoptotic crosstalk, connecting with cytotoxic effector pathways (e.g., perforin/granzymes) that drive keratinocyte death. This aligns with robust evidence identifying secreted GNLY from cytotoxic T cells and NK cells as a principal mediator of widespread keratinocyte apoptosis in severe immune-mediated eruptions, most notably the SJS/TEN spectrum, which overlaps clinically and mechanistically with EM (Subramani NK et al, 2024). Depletion or neutralization of GNLY reduces keratinocyte apoptosis, highlighting its causal role upstream of epidermal injury (Wang J et al, 2022). Our PPI results therefore recapitulate a GNLY-centric network that is biologically plausible for EM and consistent with the broader literature on immune cytotoxic mediators in EM/SJS/TEN.

Computational docking has increasingly been applied in dermatologic immunology to prioritize small molecules against apoptosis and inflammation targets; however, most prior work has focused on pathways such as Fas/FasL, TNF-α, or granzymes, with far fewer studies directly interrogating GNLY as a druggable node in EM (Viard-Leveugle I et al, 2013; Yadalam PK et al, 2025; Kocaaga A & Kocaaga M 2025; Pan RY et al, 2019; Sah PM et al, 2025). Reviews and methodological papers emphasize docking as a rapid triage tool to rank chemical libraries and guide follow-up validation, yet concrete GNLY-targeted docking reports in EM remain scarce (Fateen NK et al, 2025; Senthil R et al, 2026; Lalan BK et al, 2015; Tessema FB et al 2024). Against this backdrop, our docking of *Tacca integrifolia* phytochemicals directly to GNLY helps fill a methodological gap by moving beyond pathway-level inference to target-centric inhibition hypotheses.

All four *T. integrifolia* metabolites showed favorable GNLY interactions (–3.4 to –6.8 kcal/mol), with Betulinic acid yielded the strongest docking score, followed by Taccalin and Guaijaverin. While absolute docking values are not directly comparable across studies due to protocol differences, our affinity range is in line with exploratory natural-product screens that nominate sub-micromolar to low-micromolar hypotheses for immune targets. Crucially, our lead triage integrated drug-likeness and ADMET (including BBB non-permeability and predicted low toxicity), an approach consistent with best practices to reduce false positives from docking alone. This integrated selection pipeline mirrors contemporary *in-silico* dermatology workflows and strengthens the translational plausibility of our shortlisted compounds.

The pharmacologic profile of *T. integrifolia* supports anti-inflammatory and analgesic actions in vivo, and prior *in-silico* work on its constituents has reported drug-like ADMET features—context that lends biological plausibility to our GNLY docking results (Maliwong J et al, 2023; Yi JE at al, 2010). Betulinic acid, documented within *T. integrifolia*, has been repeatedly associated with immunomodulatory and cytoprotective activities across models, suggesting a mechanistic rationale for mitigating GNLY-driven epithelial injury (Pashmina S et al, 2024; Oliveira-Costa JF et al, 2022). Together, these data support *T. integrifolia* as a credible natural reservoir for GNLY-modulating scaffolds and justify advancing the top hits for deeper validation.

EM is an acute, immune-mediated mucocutaneous disorder in which cytotoxic lymphocyte mediators (GNLY among them) are implicated in keratinocyte death, similar to the SJS/TEN continuum. Current treatment options for erythema multiforme primarily rely on corticosteroids, antiviral agents, and systemic immunosuppressants (Soares A et al 2021). Although effective in selected cases, these therapies are associated with adverse effects and lack specificity toward key mediators of keratinocyte apoptosis. In contrast, GNLY-directed modulation may offer a more targeted approach by intervening upstream in the cytotoxic cascade responsible for epithelial injury. By nominating drug-like, non-BBB-permeant phytochemicals with predicted GNLY inhibition, our findings dovetail with the therapeutic need for targeted, safer systemic options beyond corticosteroids and non-specific

immunosuppressants. The GNLY-centric strategy is congruent with recent updates in SJS/TEN biology that elevate GNLY as a pivotal effector; although EM differs in trigger profile and severity, shared effector mechanisms argue for cross-applicability of GNLY-directed interventions.

LIMITATIONS & FUTURE DIRECTION:
The present study has certain limitations inherent to computational approaches. Docking predictions may be influenced by factors such as scoring-function bias, limited receptor flexibility, and the possibility of multiple binding poses. In addition, the dynamic behavior of GNLY, including its oligomerization and interactions with cellular membranes, may not be fully captured in a static modeling environment. Therefore, further studies are warranted to validate these findings. Future work should focus on molecular dynamics simulations to assess the stability of protein–ligand complexes and the persistence of key residue interactions, along with binding free energy calculations (e.g., MM/PBSA or MM/GBSA) for the identified lead compounds. Experimental validation through in vitro assays, such as evaluating keratinocyte apoptosis in GNLY-mediated systems and studying GNLY–ligand interactions, will be essential. Additionally, assessing selectivity against related cytotoxic proteins is important to minimize potential off-target effects. Finally, ex vivo studies using patient-derived samples or cytotoxic lymphocytes would provide clinically relevant insights and help bridge the gap between computational predictions and biological outcomes.

Conclusion

This study demonstrates that phytochemicals derived from *Tacca integrifolia* may serve as potential inhibitors of granulysin (GNLY), a key mediator of keratinocyte apoptosis in erythema multiforme. Protein–protein interaction analysis reinforced the central role of GNLY in immune and apoptotic signaling pathways, while interaction analysis indicated favorable binding profiles of the selected compounds. Among them, taccalin, betulinic acid, and γ-aminobutyric acid emerged as the most promising candidates based on their interaction strength, drug-likeness, and favorable pharmacokinetic properties, including lack of blood–brain barrier permeability and low predicted toxicity. Collectively, these findings suggest that *Tacca integrifolia* represents a promising natural source for identifying GNLY-targeted modulators and provides a potential direction for developing more specific therapeutic strategies in erythema multiforme; however, further validation through molecular dynamics simulations and experimental studies is necessary to confirm their efficacy and clinical relevance.

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Nil

Statement of Ethics:
Ethical approval is not applicable as it is an invitro study.

Conflict of Interest Statement:
The authors have no conflicts of interest to declare.

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