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Network pharmacology-guided probiotic metabolite therapy for acne vulgaris to target *Cutibacterium acnes* CAMP factors through *Pediococcus acidilactici* BCBH1 fatty acids

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Abstract
Acne vulgaris is a chronic inflammatory skin disorder predominantly caused by *Cutibacterium acnes* and its virulence-associated CAMP (Christie-Atkins-Munch-Petersen) factors, particularly CAMP1 and CAMP2, which contribute to inflammation and bacterial survival. With increasing antibiotic resistance and concerns over microbiome disruption from conventional treatments, probiotic-derived postbiotics present a promising alternative. This study aimed to investigate the anti-acne potential of fatty acids produced by *Pediococcus acidilactici* BCBH1, targeting CAMP1 and CAMP2 proteins of *C. acnes* using a network pharmacology-guided approach. Metabolite profiling via GC-MS identified vaccenic acid as a major fatty acid metabolite

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(4.88 mg/L at 48 h under 10% linoleic acid stress). Virulence prediction confirmed high pathogenicity of CAMP1 (score 0.9055) and CAMP2 (score 0.9927). Molecular docking revealed strong binding affinities of vaccenic acid to CAMP1 and CAMP2 with binding energies of –9.6 kJ/mol and –9.3 kJ/mol, respectively, outperforming traditional anti-acne compound 4-terpineol (–9.0 kJ/mol and –8.7 kJ/mol). Molecular dynamics simulations further validated the stable interaction of vaccenic acid with CAMP proteins over 100 ns. Pharmacokinetic analyses indicated vaccenic acid's favorable absorption and safety profiles with no blood–brain barrier permeability. These findings highlight vaccenic acid as a potent, microbiome-friendly therapeutic candidate for acne management. Future work should focus on experimental validation, formulation development, and combinatorial strategies to enhance clinical efficacy and safety.

Introduction

Acne vulgaris is a common chronic inflammatory skin condition that predominantly affects adolescents and young adults, although it can persist or even begin in adulthood.[1] Characterized by the formation of comedones, papules, pustules, nodules, and cysts, acne typically occurs in sebaceous gland–rich areas such as the face, back, and chest.[2] The condition is multifactorial in origin, involving excess sebum production, follicular hyperkeratinization, colonization by specific skin microbiota, and inflammation. While not life-threatening, acne significantly impairs quality of life and can lead to long-term psychological effects, including low self-esteem, social withdrawal, and depression.[3] Its prevalence and psychosocial burden have made acne vulgaris a major concern in dermatology, prompting ongoing efforts to identify more effective and sustainable treatment modalities.[4] Globally, it is estimated that more than 85% of adolescents experience some degree of acne, making it one of the most widespread dermatological disorders. Conventional therapies such as topical retinoids, benzoyl peroxide, and systemic antibiotics often provide temporary relief but are associated with side effects and increasing antimicrobial resistance. Hormonal therapies and isotretinoin, though effective, require careful monitoring due to their potential systemic risks. These challenges have intensified the search for alternative therapeutic strategies, including probiotic-derived metabolites and natural compounds with anti-inflammatory or antimicrobial properties.

Among the numerous factors implicated in acne vulgaris, *Cutibacterium acnes* (formerly *Propionibacterium acnes*) plays a pivotal pathogenic role. This gram-positive, anaerobic bacterium is a dominant member of the skin microbiome, particularly in sebaceous environments. Although it exists as a commensal under healthy conditions, dysbiosis or strain-level differences can trigger pathogenic behavior.[5] Virulence factors secreted by *C. acnes*, including lipases, proteases, hyaluronate lyase, and notably the Christie–Atkins–Munch-Petersen (CAMP) factor proteins, facilitate tissue invasion, immune activation, and inflammation. CAMP factors 1 (CAMP1) and 2 (CAMP2) have been identified as key proinflammatory proteins that disrupt epithelial integrity and enhance bacterial survival by interacting with host immune components. These proteins stimulate toll-like receptor (TLR)-2-mediated pathways, leading to the overproduction of inflammatory cytokines such as IL-1β, IL-8, and TNF-α. This cascade not only exacerbates inflammation but also contributes to follicular damage, ultimately manifesting as clinical acne lesions.[6,7]

Conventional acne therapies range from topical retinoids (e.g., tretinoin, adapalene, tazarotene) and benzoyl peroxide to systemic antibiotics (e.g., tetracycline, doxycycline, minocycline, erythromycin, clindamycin), hormonal agents (e.g., oral contraceptives, spironolactone, cyproterone acetate), and isotretinoin. While these treatments can be effective, they are often associated with considerable limitations.[8] Topical agents frequently cause dryness, erythema, and skin irritation, while long-term use of antibiotics fosters antimicrobial resistance and disrupts the skin microbiota.[9] Hormonal therapies are limited to specific populations and carry endocrine-related side effects. Isotretinoin, though potent, is linked with severe teratogenic risks, mucocutaneous adverse effects, and psychiatric symptoms. These drawbacks have necessitated the development of alternative therapeutic strategies that are both effective and better tolerated.[10] Increasing resistance to antibiotics and growing consumer demand for natural and sustainable dermatological solutions have particularly intensified interest in biologically-based therapies.[11] Probiotics have emerged as a promising frontier in acne management due to their ability to restore microbial balance and exert anti-inflammatory effects.[12] Among them, *Pediococcus acidilactici* BCBH1, a probiotic strain originally isolated from Tibetan Kefir, has demonstrated significant potential.[13] When cultured in linoleic acid (LA)-enriched media, this strain metabolizes the fatty acid into bioactive derivatives, including vaccenic acid, which have shown antibacterial and anti-inflammatory properties. These fatty acid metabolites are capable of modulating host–pathogen interactions by disrupting bacterial virulence mechanisms, such as those mediated by CAMP factor proteins of *C. acnes*.[14] Unlike traditional treatments, probiotic-based therapies offer a dual advantage, that is, rebalancing skin microbiota while minimizing side effects. Thus, probiotic-derived fatty acids represent a novel, nontoxic approach to attenuating *C. acnes* pathogenicity and managing acne vulgaris in a microbiome-friendly manner.[15]

This study aims to investigate the therapeutic potential of fatty acid metabolites produced by *P. acidilactici*

BCBH1 in targeting the virulence of *C. acnes*, particularly through the inhibition of its CAMP factor proteins, using a network pharmacology-guided approach. The methodology involves culturing *P. acidilactici* BCBH1 in media enriched with varying concentrations of LA to induce metabolite production, followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis for metabolite profiling. Key fatty acids identified were further evaluated for their interaction with virulence-associated proteins of *C. acnes*, with a focus on vaccenic acid. Using tools such as protein–protein interaction network construction, hub gene analysis, and molecular docking, this study integrates computational and experimental techniques to elucidate the mechanistic role of probiotic-derived fatty acids. The ultimate objective is to establish a safe, effective, and targeted probiotic metabolite therapy for acne vulgaris, offering a viable alternative to conventional treatment modalities.

Methodology

Bacterial isolation and GC-MS analysis

In our previously established study, *P. acidilactici* BCBH1 with an NCBI accession ID GCF_021568595.1, a strain originally isolated from Tibetan Kefir, was retrieved from the Dairy Laboratory Culture Bank at Beijing Technology and Business University, China, and subcultured in MRS medium at 37 °C.[16] For experimental setup, 1.0 mL of inoculum (9.0 log CFU/mL) was introduced into LA-MRS medium across 10 flasks containing varying concentrations of LA, from 1 to 10% (w/v). Cultures were incubated at 37 °C for 48 h, and samples were collected at 0, 8, 16, 24, 36, and 48 h to monitor viable cell counts and fatty acid metabolite production.

Fatty acid metabolites were first quantified using spectrophotometry. The samples were centrifuged to obtain the supernatant, which was then mixed with isopropanol and hexane for lipid extraction. The hexane phase was separated, purified, and analyzed at 233 nm to estimate the concentration of lipid-derived metabolites. For GC-MS analysis, the fatty acids were further processed by adding heptadecanoic acid (C17:0) as an internal standard. Lipids were extracted from the supernatant using isopropanol and n-hexane, followed by vortexing, phase separation, and drying under liquid nitrogen to prepare for analysis.

The resulting fatty acid methyl esters (FAMEs) were analyzed using a Shimadzu GC-2010 gas chromatograph coupled with a mass spectrometer. Injection was performed in split mode (10:1) at 250 °C using helium as the carrier gas. Chromatographic separation was achieved on a TR-Wax MS polar capillary column. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, scanning a mass range of 33–450 m/z. Fatty acid metabolites were identified by comparing the obtained mass spectra with those in the NIST 2014 library. Statistical analysis of the data was conducted using analysis of variance (ANOVA) in SAS software (version 9), with Duncan's multiple range test used to determine significant differences ($P \leq 0.05$). Results were reported as mean ± standard deviation.

Virulence prediction of CAMP factor proteins of Cutibacterium acnes

Based on the literature evidence highlighting the pivotal role of CAMP factor proteins in the pathogenesis of *C. acnes*, particularly in acne vulgaris,[17] the protein sequences of CAMP factor 1 (CAMP1) and CAMP factor 2 (CAMP2) were retrieved from the UniProt database. These sequences were analyzed using VirulentPred (http://bioinfo.icgeb.res.in/virulent/), a support vector machine (SVM)-based web server developed for predicting bacterial virulent proteins. The analysis was conducted in amino acid sequence input mode, with the SVM prediction score ranging from 0 to 1. The cutoff or threshold value was set at 0.5. According to the server's classification criteria, a score equal to or above 0.5 indicates that the protein is predicted to be virulent, whereas a score below 0.5 suggests nonvirulence. The server outputs a binary classification (virulent/nonvirulent) along with the associated prediction score for each input protein. This analysis provided computational evidence supporting the virulent nature of CAMP1 and CAMP2 proteins, thereby confirming their potential role in host-pathogen interactions and inflammatory responses associated with acne vulgaris.

Protein-protein network construction and visualization

The potential interactions and functional associations of the CAMP factor proteins of *C. acnes* were explored through protein-protein interaction (PPI) network using the STRING database (Search Tool for the Retrieval of Interacting Genes/Proteins, https://string-db.org/). The protein sequences of CAMP1 and CAMP2 were submitted under the organism *C. acnes*, and the interaction network was generated with a confidence score cutoff set to medium (≥ 0.4) to ensure reliable associations. The resulting PPI network was visualized to identify potential interacting partners and assess their involvement in biologically relevant pathways. This analysis, in combination with functional annotation, provided insights into the molecular interactions and potential roles of CAMP factor proteins in the pathogenesis of acne vulgaris.

Identification of hub genes

To determine the most influential genes within PPI network of *C. acnes* associated with acne vulgaris, hub gene analysis was performed using Cytoscape software (version 3.9.1) equipped with the cytoHubba plugin. The CAMP factor protein-derived PPI network, previously constructed using the STRING database, was imported into Cytoscape for further topological analysis. Within cytoHubba, multiple algorithms were available for hub gene ranking; among them, the Maximal Clique Centrality (MCC) algorithm was selected due to its proven sensitivity and accuracy in identifying essential hub genes. The top 10 hub genes were extracted based on their MCC scores, which reflect their level of connectivity and centrality within the network.

These hub genes are considered to play crucial roles in the molecular pathogenesis of acne vulgaris and were selected for downstream metabolite interaction analysis.

Network pharmacological analysis

To investigate the therapeutic relevance of the identified hub genes, a network pharmacology approach was adopted by integrating the top 10 hub genes with fatty acid metabolites identified through GC-MS analysis of *P. acidilactici* BCB1H. The goal was to determine which metabolite(s) exhibited potential for targeting the pathogenic mechanisms of *C. acnes* involved in acne vulgaris. The analysis was performed using Cytoscape (v3.9.1), where hub genes and GC-MS-derived metabolites were used as nodes, and their possible interactions were mapped. The network was constructed with interaction edges defined by molecular docking results or known gene–compound associations, and the significance of each interaction was evaluated based on confidence score thresholds (≥ 0.7 for high-confidence interactions) and P-values ($P \leq 0.05$). These thresholds ensured that only statistically significant and biologically meaningful interactions were included in the final analysis. The network topology was visualized to identify the metabolite exhibiting the highest degree of interaction with key hub genes, thereby highlighting its potential as a therapeutic candidate for modulating the virulence of *C. acnes* in acne vulgaris.

Protein 3D structure and fatty acid metabolite retrieval

Network pharmacological analysis identified vaccenic acid as the most interactive fatty acid metabolite with the pathogenic hub genes of *C. acnes*, particularly with the CAMP factor proteins, which are known to play a significant role in the virulence of *C. acnes*. To further investigate this interaction, the three-dimensional (3D) structures of the CAMP factor proteins, CAMP1 and CAMP2, were retrieved from the AlphaFold Protein Structure Database, which provides high-accuracy protein models generated using deep learning techniques. The 3D structure of vaccenic acid was retrieved in .sdf and .pdb formats from the PubChem compound database for molecular docking studies. For comparative analysis, a conventional anti-acne compound, 4-terpineol, commonly found in tea tree oil and widely used in acne treatment, was also retrieved from PubChem. These molecular structures were prepared for docking to analyze binding affinities and interaction profiles with the CAMP factor proteins, enabling a comparative evaluation of vaccenic acid's therapeutic potential.

Molecular docking analysis

Molecular docking analysis was performed using AutoDock Vina to evaluate the binding affinities and molecular interactions between the fatty acid metabolite vaccenic acid and the virulence-associated CAMP factor proteins (CAMP1 and CAMP2) of *C. acnes*. Before docking, all ligand and protein structures were energy-minimized and converted into PDBQT format using AutoDock Tools. Grid box parameters were defined to encompass the entire active site region of the protein, with grid spacing set to 1.0 Å. The exhaustiveness parameter was kept at 8 to ensure thorough conformational sampling. The binding affinity (kcal/mol) and interaction types, that is, hydrogen bonding and hydrophobic interactions, were analyzed post-docking to evaluate the strength and specificity of ligand–protein binding. The results were visualized using Discovery Studio Visualizer.

Toxicity analysis

The toxicity profiles of both vaccenic acid and the reference drug 4-terpineol were evaluated using SwissADME, an online tool for assessing pharmacokinetics, drug-likeness, and medicinal chemistry friendliness. The canonical SMILES of both compounds were entered into the SwissADME interface. Parameters, including Lipinski's Rule of Five, bioavailability score, gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, and predicted PAINS alerts were considered to assess drug-likeness. Additionally, the BOILED-Egg model was used to visualize passive GI absorption and brain access prediction.

Molecular dynamic simulation analysis

Molecular dynamic (MD) simulations of the docked complexes of CAMP1–vaccenic acid and CAMP2–vaccenic acid were conducted using GROMACS 2021. The CHARMM36 force field was employed for protein topology generation, and ligand topologies were generated using the CGenFF server. Each complex was solvated in a cubic box with a 1.0 nm distance from the edges and filled with SPC/E water molecules. The system was neutralized with Na^+ or Cl^- ions as needed. Energy minimization was carried out using the steepest descent algorithm for 50,000 steps. The systems were equilibrated under NVT (constant Number of particles, Volume, and Temperature) for 100 ps and then under NPT (constant Number of particles, Pressure, and Temperature) for 100 ps. The production MD run was conducted for 100 ns at 300 K and 1 atm pressure. Post-simulation, the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bonding analysis were computed to assess the structural stability and interaction dynamics of the protein–ligand complexes.

Results

Bacterial isolation and GC-MS analysis

In our previously published study,[16] *P. acidilactici* BCBH1 was evaluated for its tolerance to increasing concentrations of LA ranging from 1 to 10% (w/v) in MRS broth. The strain exhibited consistent and robust growth across all LA concentrations, with a characteristic growth curve showing logarithmic increase up to 24 h, a stationary phase until

48 h, and a subsequent decline by 72 h. Notably, higher LA concentrations were associated with a slight enhancement in growth, suggesting a potential adaptation mechanism. While many lactic acid bacteria are sensitive to LA even at low concentrations (e.g., 25 µg/mL), *P. acidilactici* BCBH1 demonstrated exceptional resilience, thriving even at 10% (w/v) LA. The increased optical density (OD_{600}) readings at elevated LA concentrations indicated metabolic conversion of free LA into less toxic fatty acid derivatives, particularly during the stationary phase, suggesting a detoxification response.

Fatty acid metabolites produced at various LA concentrations and time intervals (8, 16, 24, 48, and 72 h) were subsequently analyzed using GC-MS. Thirteen distinct metabolites were identified, including 11 LA-derived analogs such as cis-vaccenic acid, linoelaidic acid, octanoic acid, and (Z,Z,Z)-8,11,14-eicosatrienoic acid, as well as a phenolic compound and a fatty alcohol. Quantitative profiling revealed metabolite concentrations ranging from 0.1 to 19.18 mg/L, with cis-vaccenic acid notably present at 4.88 mg/L under 10% LA, highlighting its prominent production under high LA stress. These findings confirm the capacity of *P. acidilactici* BCBH1 to not only tolerate but also metabolically convert LA into functionally significant derivatives.

Virulence prediction of CAMP factor proteins of *Cutibacterium acnes*

The virulence potential of the CAMP factor proteins of *C. acnes* was assessed using the VirulentPred server, which utilizes an SVM-based algorithm for prediction. The protein sequence of CAMP1, identified by NCBI Accession ID APH07537, exhibited a high virulence score of 0.9055, while the CAMP2 protein, with NCBI Accession ID CAM4120460, demonstrated an even higher virulence score of 0.9927. According to VirulentPred, scores range from 0 to 1, with a threshold cutoff of 0.5, above which proteins are considered virulent. Both CAMP1 and CAMP2 proteins exceeded this threshold significantly, indicating their strong virulent nature. These results support existing literature that highlights the crucial role of CAMP factor proteins in the pathogenicity of *C. acnes* and their involvement in acne vulgaris development.

Protein-protein network construction and visualization

According to STRING network data, the CAMP factor proteins of *C. acnes*, specifically CAMP1 (ALT35373.1) and

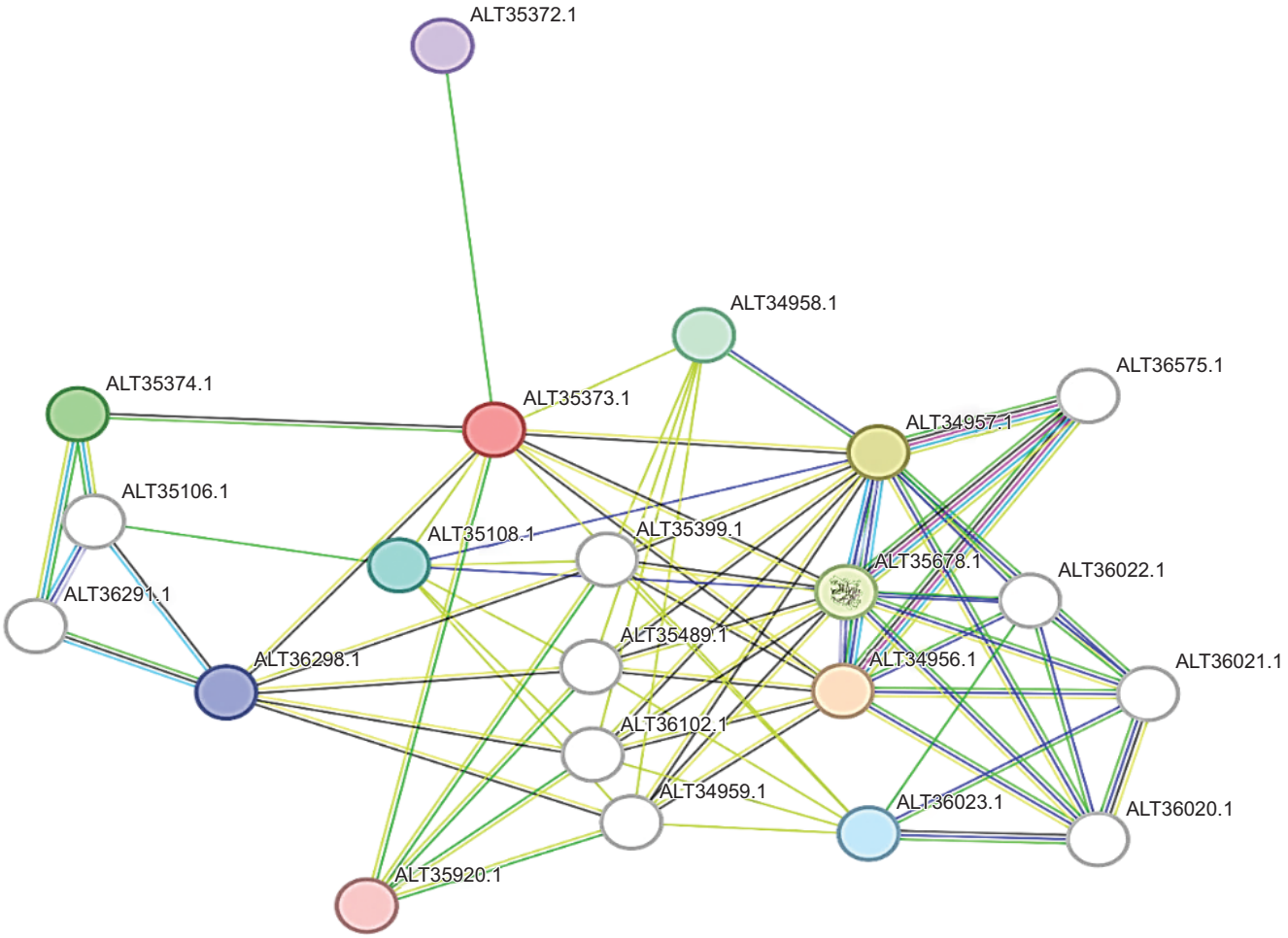

Figure 1 STRING network interaction map of CAMP1 (ALT35373.1) and CAMP2 (ALT34956.1) proteins of *Cutibacterium acnes* causing acne vulgaris.

CAMP2 (ALT34956.1), are both implicated in the pathogenesis of acne vulgaris and share a functional role as virulence factors that promote inflammation and immune evasion. Each of these proteins displays a node degree of 11, indicating that they interact with 11 other proteins in the STRING interaction network. This moderate but significant level of connectivity suggests that both CAMP1 and CAMP2 are involved in coordinated functional roles within the bacterial proteome, particularly in mechanisms that facilitate host colonization and inflammatory stimulation (Figure 1).

Functionally, CAMP2 is annotated as a sialidase enzyme, while CAMP1, though not directly annotated with enzymatic functions in the STRING data, appears in the same functional clusters as CAMP2, indicating a closely related or synergistic role. STRING's molecular function annotations for CAMP2 include exo-alpha-sialidase activity (GO:0004308), hydrolase activity (GO:0016787), and hydrolase activity acting on glycosyl bonds (GO:0016798). These enzymatic capabilities allow CAMP2 to cleave sialic acid residues from host glycoproteins and glycolipids, a process critical for *C. acnes* to penetrate the skin barrier, evade host immune responses, and initiate inflammation. While CAMP1 lacks specific sialidase GO terms, its co-clustering and co-occurrence with CAMP2 in functional modules strongly suggest a complementary role, possibly as a cofactor or structural partner involved in virulence expression. From a biological process standpoint, CAMP2 is linked through STRING to carbohydrate metabolic processes (GO:0005975), primary metabolic processes (GO:0044238), and organic substance metabolism (GO:0071704). These functions are essential for survival in the lipid- and nutrient-rich environment of the pilosebaceous follicle, where *C. acnes* thrive. By metabolizing host glycoconjugates, CAMP2 supports bacterial growth and contributes to the local accumulation of proinflammatory metabolites. Though CAMP1 is not annotated with these GO terms individually, its coexpression and network proximity suggest its involvement in similar metabolic and immunomodulatory functions.

STRING functional cluster data provides additional insight into the virulence profile of both CAMP1 and CAMP2. These proteins are part of clusters such as CL:3439 (alpha-sialidase activity and CAMP factor) and CL:3436 (CAMP factor and alpha-sialidase related proteins), which also include lipases and metal ion transporters. These clusters reflect functional cooperation among bacterial factors that degrade host tissue, manipulate the immune environment, and extract nutrients, that is, all hallmarks of *C. acnes* virulence. Furthermore, Reactome pathway mapping links CAMP2 (and by inference, CAMP1) to the innate immune system (MAP-168249), supporting their involvement in triggering host immune reactions. Both CAMP1 and CAMP2 contribute to immune system activation, particularly through the induction of proinflammatory cytokines such as IL-1β and TNF-α, which drive the formation of inflammatory acne lesions, including papules and pustules. CAMP2 is specifically mapped to KEGG pathways involved in other glycan degradation (cacn00511), sphingolipid metabolism (cacn00600), and general metabolic pathways (cacn01100), all of which underscore its role in modulating the cutaneous microenvironment and enhancing *C. acnes* pathogenicity.

STRING database analysis of CAMP1 and CAMP2 highlights their shared role as virulence factors of *C. acnes* in the development of acne vulgaris. Both proteins show evidence of interaction with other metabolic and immune-modulating proteins, with CAMP2 having defined enzymatic functions and CAMP1 likely acting as a structurally or functionally supportive factor. Their network integration, pathway associations, and induction of inflammatory cascades position them as critical contributors to acne pathophysiology. As such, targeting CAMP1 and CAMP2 together may offer a promising therapeutic strategy to mitigate *C. acnes*-induced inflammation while preserving the balance of the skin microbiome.

HUB genes identification

A comprehensive hub gene analysis was conducted to elucidate the key molecular players involved in the pathogenesis of acne vulgaris caused by *C. acnes*. Initially, 20 proteins identified through STRING interaction analysis were subjected to further examination using the CytoHubba plugin in Cytoscape to determine their topological importance in the interaction network. Among these, the top 10 hub genes were prioritized based on the Maximal Clique Centrality (MCC) method, which identifies essential nodes by evaluating their participation in highly interconnected subnetworks (Figure 2).

The analysis revealed that CAMP1 (ALT35373.1) and CAMP2 (ALT34956.1) are the top-ranked hub proteins, with MCC scores of 178 and 156, respectively. These values signify their centrality in maintaining network structure and function, especially in relation to virulence and immune interactions associated with acne. Notably, CAMP1 exhibited the highest degree centrality of 13, reflecting its extensive direct interactions with other proteins. It also demonstrated a high EPC value of 12.548, and a betweenness centrality of 45.27, indicating its crucial role as a mediator of communication within the protein network. These metrics collectively suggest that CAMP1 serves as a key regulatory and signaling hub. Similarly, CAMP2 displayed a degree of 11, an EPC value of 12.047, and a betweenness centrality of 26.97, further reinforcing its importance in the structural integrity and functional dynamics of the *C. acnes* interaction network. Its central position suggests significant involvement in processes such as host immune modulation, bacterial adhesion, and inflammatory triggering, all hallmarks of acne pathophysiology.

In addition to CAMP1 and CAMP2, other notable proteins were identified. ALT35678.1 and ALT36022.1 followed in ranking with MCC scores of 132 and 128, respectively, marking them as potentially important secondary nodes in the network. Proteins such as ALT35374.1 (Degree: 3, EPC: 5.093, Betweenness: 7.04), ALT35106.1 (Degree: 4, EPC: 6.389, Betweenness: 8.56), and ALT36023.1 (Degree: 8, EPC: 11.003) also demonstrated substantial integrative and connective roles, highlighting a broader network of interactions that support the pathogenic potential of *C. acnes*. The hub gene analysis underscores CAMP1 and CAMP2 as the core regulatory nodes in the *C. acnes* protein–protein interaction network, playing pivotal roles in virulence, immune evasion, and inflammatory response.

Network pharmacological analysis

A comprehensive network pharmacological analysis was conducted to elucidate the interactions between bacterial fatty acid metabolites derived from *P. acidilactici* BCBH1 (identified via GC-MS analysis) and hub genes of *C. acnes* implicated in acne vulgaris. The tripartite interaction network was constructed using Cytoscape, incorporating 12 bacterial metabolites, 2 CAMP factor proteins (CAMP1 and CAMP2) from *C. acnes*, and the hub genes identified via CytoHubba analysis (Figure 3). Among the analyzed metabolites, vaccenic acid emerged as the most significant bioactive compound, demonstrating the strongest interaction with both CAMP factor proteins. It showed a confidence score of 0.92 (P = 0.04) with CAMP1 and 0.90 (P = 0.03) with CAMP2, indicating statistically significant and high-affinity binding predictions. In

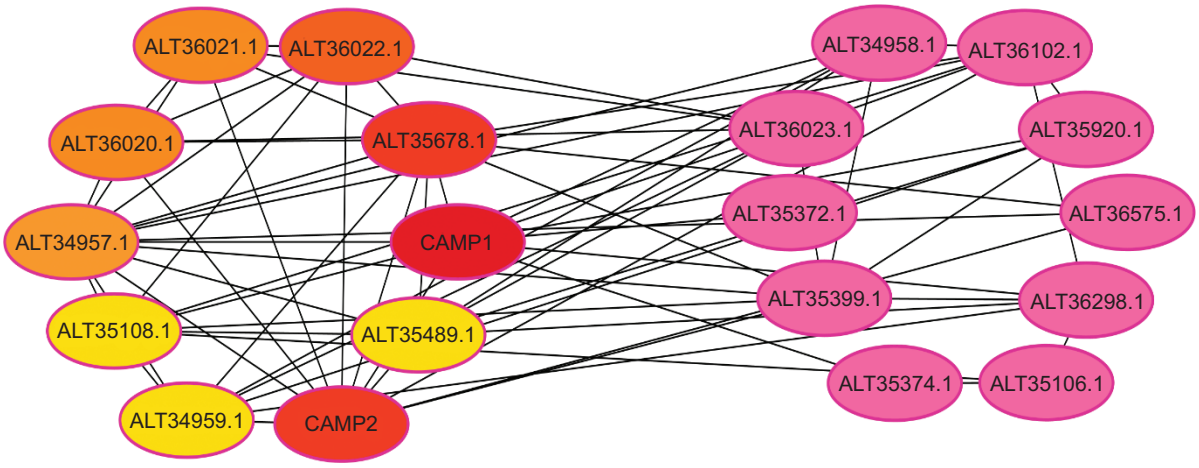

Figure 2. Hub gene network of 20 *Cutibacterium acnes* proteins identified through STRING, highlighting the top 10 Hub genes interpreted using the CytoHubba plugin in Cytoscape.

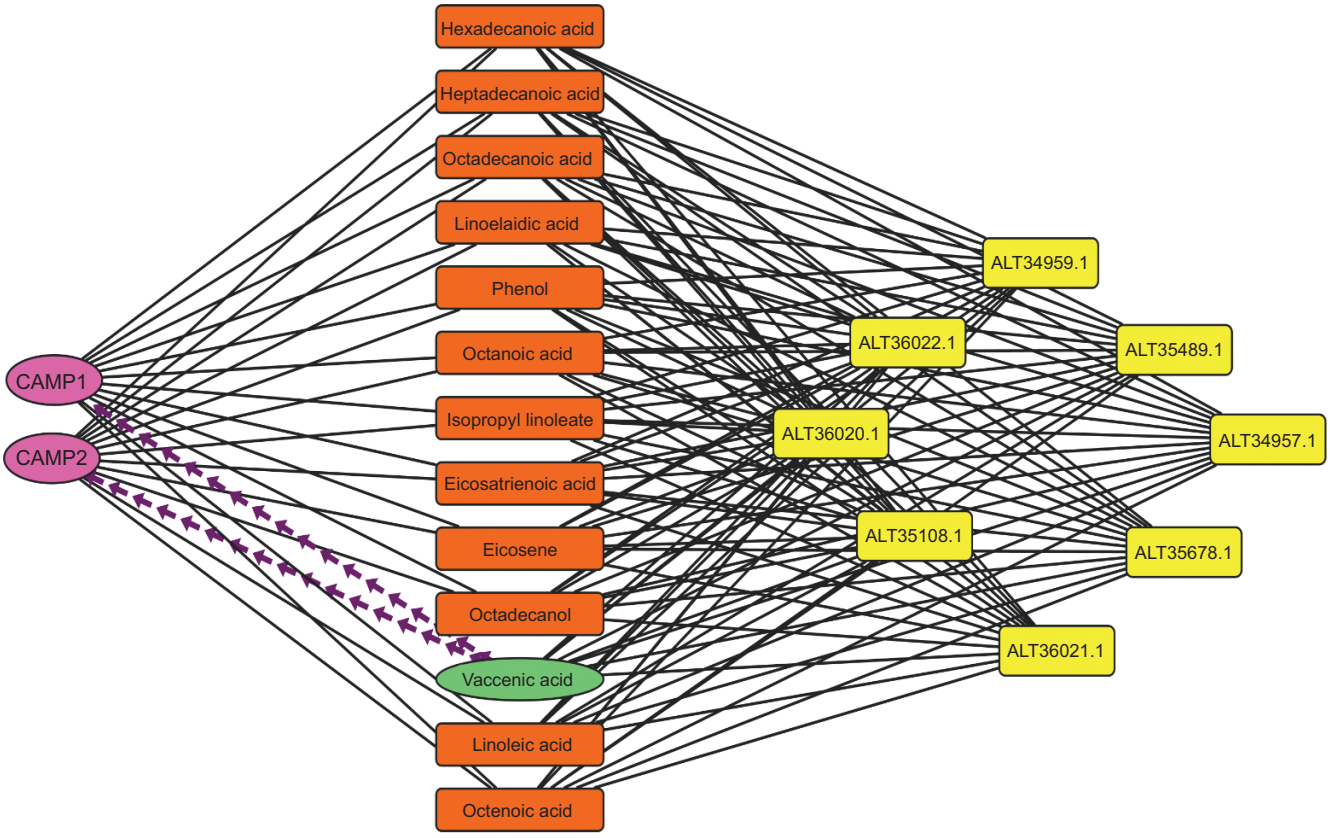

Figure 3 Pharmacological interaction network illustrating the relationships among bacterial metabolites (orange), hub genes of *Cutibacterium acnes* (yellow), and CAMP factor proteins (purple), as identified through CytoHubba analysis in Cytoscape. Vaccenic acid (green) is shown to interact at the highest confidence score with both CAMP1 and CAMP2, highlighting these virulence-associated proteins as the most significant targets.

contrast, the remaining metabolites exhibited lower interaction scores, all below 0.7, with CAMP proteins and hub genes, suggesting weaker or less relevant pharmacological associations. These findings highlight vaccenic acid as a central and potentially therapeutic metabolite with a strong predicted affinity toward key virulence factors of *C. acnes*.

Protein 3D structure and fatty acid metabolite retrieval

The 3D structures of the virulence-associated CAMP factor proteins from *C. acnes*, namely CAMP1 and CAMP2, were retrieved from the AlphaFold Protein Structure Database to facilitate structural and docking analyses. The respective AlphaFold IDs for these proteins were AF-A0A1L3MYX2-F1 for CAMP1 (Figure 4A [a]) and AF-Q5QCW8-F1 (Figure 4A [b]) for CAMP2, both offering high-confidence, AI-predicted structural models suitable for in silico interaction studies.

In parallel, key fatty acid metabolites derived from the probiotic strain *P. acidilactici* BCBH1 were identified via GC-MS analysis, with particular attention given to compounds with potential anti-acne activity. Among them, cis-vaccenic acid, a bioactive unsaturated fatty acid, was retrieved from the PubChem database using its PubChem Compound ID: 5282761 (Figure 4B [a]). Additionally, 4-terpineol, a well-known component of traditionally used anti-acne formulations such as tea tree oil, was also selected and retrieved using PubChem ID: 11230 (Figure 4B [b]).

Molecular docking analysis

Molecular docking analysis was performed to evaluate the binding affinities and interaction profiles of the virulence-associated CAMP factor proteins (CAMP1 and CAMP2) from *C. acnes* with two key ligands: vaccenic acid (a bacterial metabolite derived from *P. acidilactici* BCBH1) and 4-terpineol (a traditionally used anti-acne compound). The analysis revealed favorable binding energies and stable interaction networks, indicating the therapeutic potential of these ligands.

For CAMP1, vaccenic acid exhibited the strongest binding affinity of −9.6 kJ/mol, forming interactions with several residues: THR68, ALA72, VAL81, ILE85, VAL127, ALA130, ALA131, HIS132, PHE146, and ARG202. These interactions included hydrogen bonds, van der Waals forces, Pi-Pi stacking, and hydrophobic contacts, contributing to the ligand's stable accommodation within the binding pocket (Figure 5A). Similarly, CAMP2 showed a strong binding affinity of −9.3 kJ/mol with vaccenic acid, interacting with the residues MET61, PRO62, GLN63, ASN75, TYR76, ILE80, ILE124, ALA127, ASN128, LEU142, and ILE146. The binding was supported by a combination of hydrogen bonds, van der Waals interactions, and Pi-Pi interactions, indicating a robust and stable ligand–protein complex (Figure 5B).

In comparison, 4-terpineol demonstrated slightly lower binding affinities. With CAMP1, the binding energy was −9.0 kJ/mol, involving the residues VAL81, ILE85, VAL127, ALA131, and PHE146, primarily stabilized by hydrophobic and van der Waals interactions (Figure 5C). For CAMP2, the binding affinity was −8.7 kJ/mol, with key interactions formed at TYR76, ILE124, ALA127, ASN128, and ILE146 (Figure 5D). Vaccenic acid consistently showed higher binding affinities and a broader interaction network with both CAMP1 and CAMP2 compared to 4-terpineol. These results suggest that vaccenic acid may serve as a more potent and stable therapeutic candidate, potentially interfering with the virulence function of *C. acnes* CAMP proteins in acne vulgaris pathogenesis.

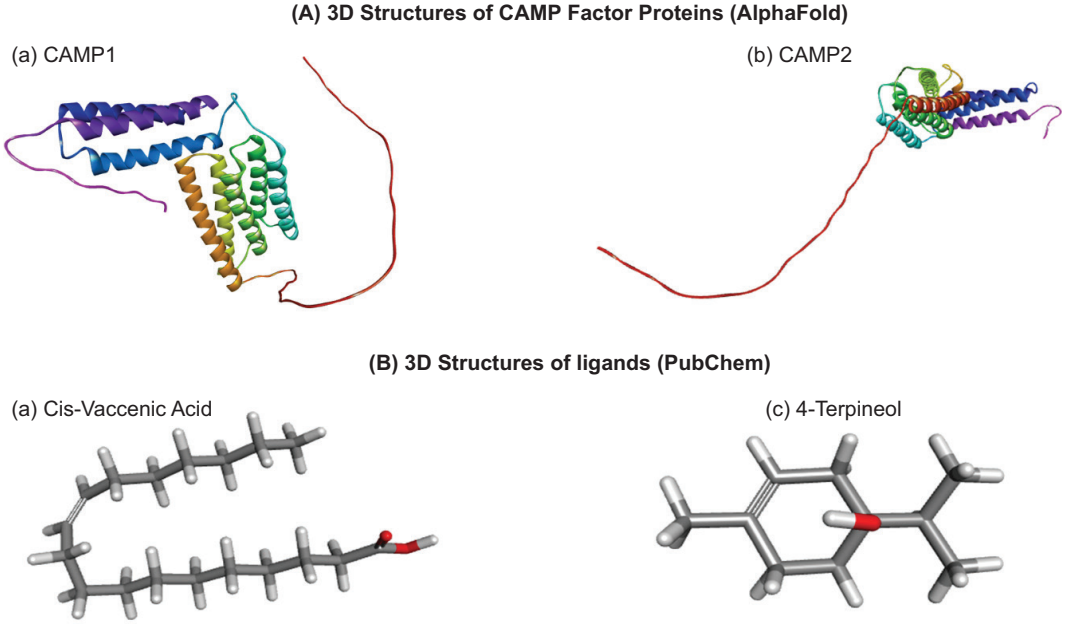

Figure 4 (A) Predicted 3D structures of *Cutibacterium acnes* CAMP factor proteins retrieved from AlphaFold: (a) CAMP1 and (b) CAMP2; (B) Chemical structures of selected ligands involved in docking studies: (a) Cis-vaccenic acid and (b) 4-terpineol.

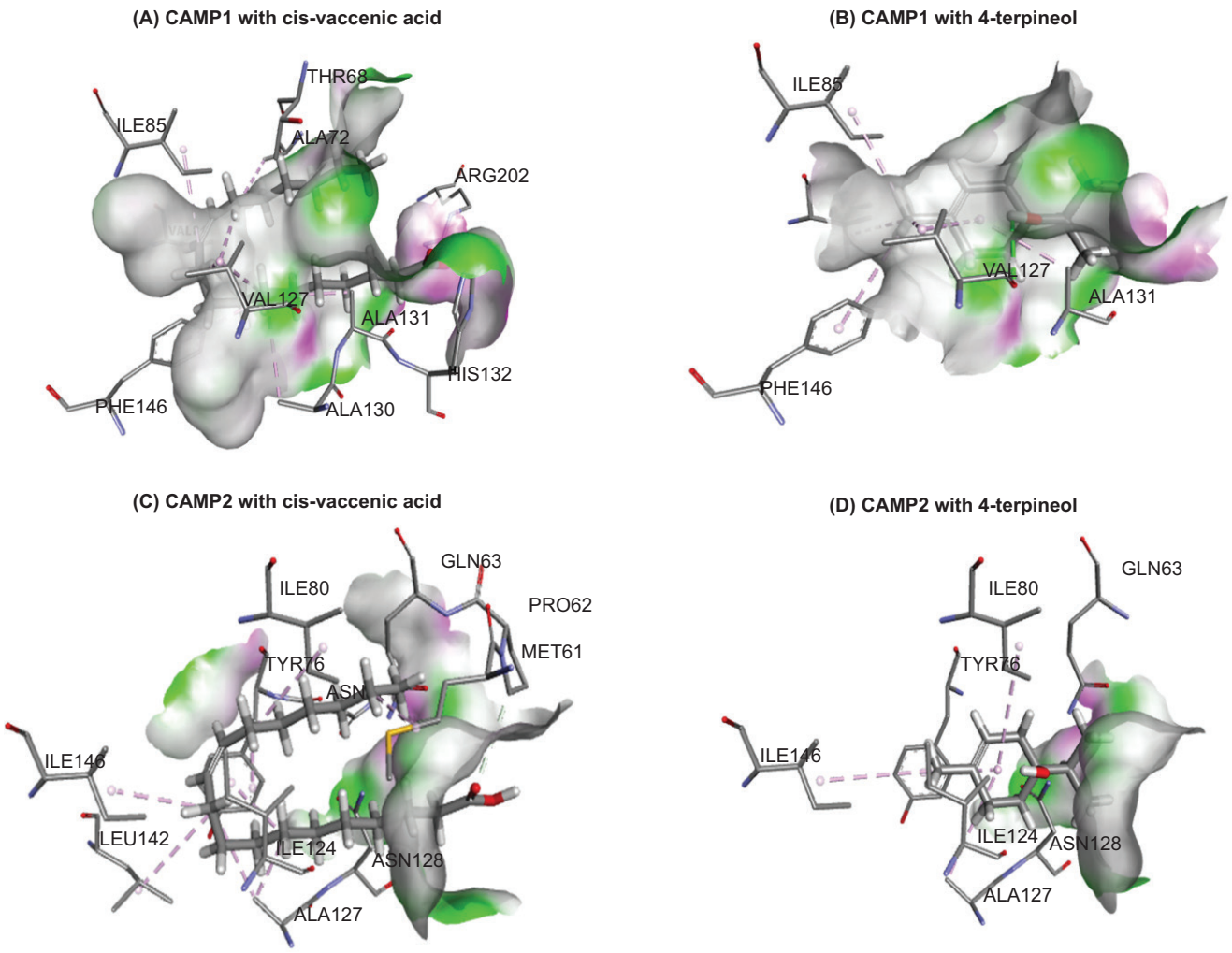

Figure 5 Molecular docking analysis of CAMP factor proteins with key ligands. (A) Docking pose of vaccenic acid bound to CAMP1 protein; (B) Docking pose of vaccenic acid bound to CAMP2 protein; (C) Docking pose of 4-terpineol bound to CAMP1 protein; (D) Docking pose of 4-terpineol bound to CAMP2 protein.

Toxicity analysis

To evaluate the safety and drug-likeness of two promising anti-acne ligands—vaccenic acid (a bacterial metabolite from *P. acidilactici* BCBH1) and 4-terpineol (a compound found in traditional herbal treatments like tea tree oil)—a comprehensive toxicity and pharmacokinetic profile analysis was conducted using the SwissADME platform. While both molecules exhibited favorable binding affinities with the virulence factors CAMP1 and CAMP2 of *C. acnes*, differences in their absorption, distribution, solubility, and potential CNS effects help determine their relative therapeutic safety (Figure 6). Vaccenic acid ($C_{10}H_{18}O_2$), with a molecular weight of 282.46 g/mol, demonstrated high gastrointestinal (GI) absorption but importantly did not cross the BBB, which significantly reduces the risk of central nervous system-related side effects. In contrast, 4-terpineol ($C_{10}H_8O$), with a lower molecular weight of 154.25 g/mol, was found to be BBB permeant, raising concerns about potential neurotoxicity or unwanted CNS interactions, particularly for a compound intended for topical or systemic dermatological use. Both molecules scored favorably under Lipinski's rule of five, with vaccenic acid showing one violation (likely due to its high number of rotatable bonds or logP), while 4-terpineol exhibited no violations, reflecting good oral bioavailability. However, vaccenic acid had a higher consensus Log P of 5.7, indicating greater lipophilicity compared to 4-terpineol's 2.6, which may affect systemic distribution and skin permeability.

In terms of solubility, 4-terpineol was consistently classified as "soluble" across multiple predictive models, with ESOL Log S = -2.78 and Ali Log S = -3.36, supporting a solubility range of 0.254–0.0675 mg/mL. Vaccenic acid, in contrast, was classified as "moderately to poorly soluble," with ESOL Log S = -5.41 (1.09E-03 mg/mL) and Silicos-IT LogSw = -5.39, indicating relatively limited aqueous solubility, which could influence formulation strategies for topical delivery. From a safety standpoint, vaccenic acid did not inhibit CYP3A4, CYP2C19, or CYP2D6, with only weak predicted inhibition of CYP1A2 and CYP2C9, while 4-terpineol showed no inhibition of any major cytochrome P450 enzymes, suggesting fewer drug-drug interaction risks.

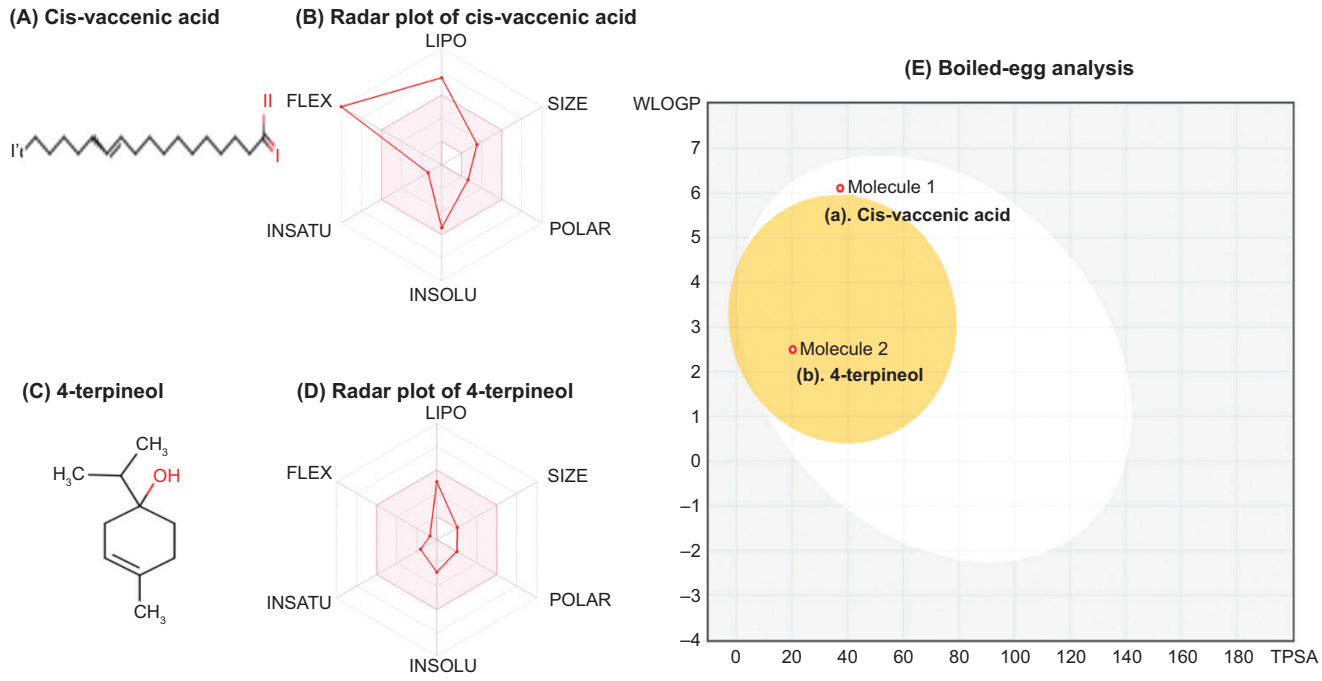

Figure 6 Toxicity analysis of cis-vaccenic acid and 4-terpineol. (A) Structure of cis-vaccenic acid; (B) Radar plot of vaccenic acid; (C) Structure of 4-terpineol; (D) Radar plot of 4-terpineol; (E) Boiled-egg analysis plot of cis-vaccenic acid and 4-terpineol.

Notably, neither compound was flagged as a P-glycoprotein (Pgp) substrate, which supports favorable cellular uptake and reduced efflux potential. Importantly, Vaccenic Acid showed no BBB permeability, a lower bioavailability score (0.55 vs 0.85), and a slightly higher synthetic accessibility score of 3.07 (compared to 4-terpineol's 3.28), indicating it is easier to synthesize and safer for non-CNS targeted applications such as acne treatment. Additionally, vaccenic acid had only one PAINS alert and one Brenk structural alert, consistent with low off-target risks. Vaccenic acid emerges as the safer and more appropriate therapeutic candidate for acne vulgaris due to its lack of BBB permeability, favorable metabolic profile, and lower risk of CNS-related toxicity. These pharmacokinetic characteristics, combined with its microbial origin and stability in docking and simulation studies, support its potential for development as a topical or systemic anti-acne agent.

Molecular dynamic simulation analysis

A 100 ns molecular dynamics (MD) simulation was performed to investigate the dynamic behavior, stability, and flexibility of the CAMP1 protein from *C. acnes* in complex with vaccenic acid, a fatty acid of potential therapeutic relevance. The simulation provided a comprehensive assessment through multiple structural and energetic analyses, affirming the stable interaction between the protein and ligand. The RMSD analysis revealed that the CAMP1 backbone achieved equilibrium after approximately 40 ns, stabilizing at an average RMSD of ~1.4 nm, indicating that the protein maintained a consistent conformation throughout the simulation. In contrast, vaccenic acid exhibited significantly lower fluctuations, with RMSD values stabilizing around 0.2–0.25 nm, suggesting that the ligand remained tightly bound within the active pocket, with minimal conformational drift (Figure 7A).

Complementary insights were obtained from the RMSF plot, which showed that the majority of residues fluctuated below 0.6 nm, with only a few terminal residues peaking near 2.5 nm, indicative of localized flexibility and largely preserved structural integrity upon ligand binding. This limited flexibility suggests that ligand interaction does not disrupt the core architecture of the CAMP1 protein (Figure 7B). Throughout the simulation, hydrogen bond analysis demonstrated sustained interactions between CAMP1 and vaccenic acid, with the number of hydrogen bonds consistently ranging between 14,000 and 16,500. This strong hydrogen bonding profile supports the hypothesis of a robust and stable protein-ligand complex (Figure 7C). Moreover, the Rg remained tightly clustered around an average of ~2.3 nm, reinforcing the overall compactness and structural stability of the complex during the simulation trajectory (Figure 7D).

Principal Component Analysis (PCA) provided further insight into the collective motions of the protein. The two principal components—PC1 and PC2—spanned ranges of -80 to +120 and -60 to +60, respectively. The relatively dispersed yet continuous nature of the PCA point cloud indicates that CAMP1 underwent essential dynamic fluctuations without major conformational transitions, suggesting a flexible yet structurally resilient behavior in the presence of vaccenic acid (Figure 7E). Additionally, the dynamic cross-correlation matrix (DCCM) analysis revealed regions of both positively correlated and anticorrelated motions among residues. A dominant diagonal yellow line confirmed internal stability through self-correlation, while localized yellow-green (positive correlation) and blue-purple

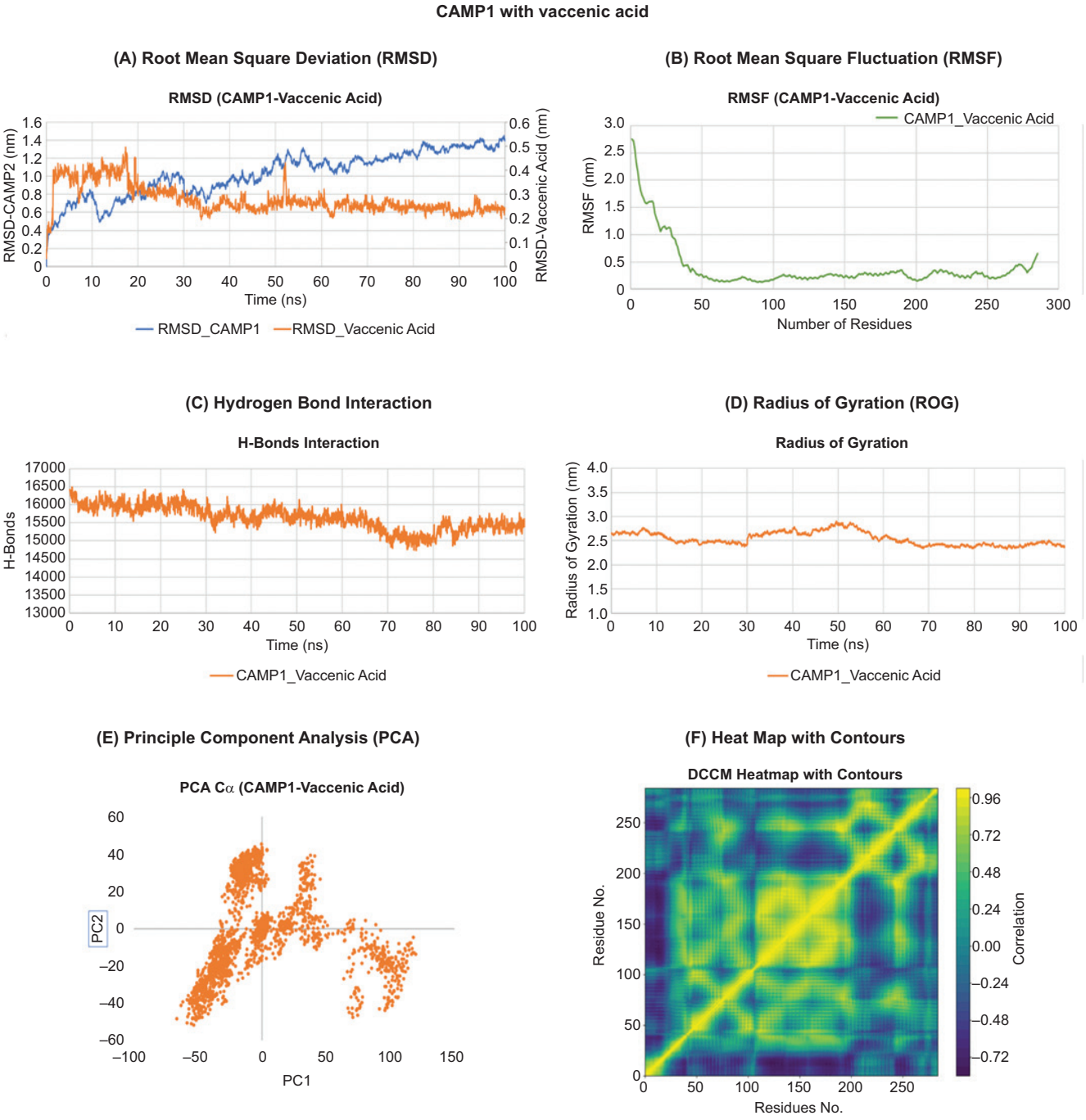

Figure 7 Molecular dynamics simulation analysis of the CAMP1-vaccenic acid complex over 100 ns. (A) RMSD plots showing stability of docked complex; (B) RMSF indicating limited residue flexibility; (C) Hydrogen bond count; (D) Radius of gyration confirming compact complex structure; (E) Principal Component Analysis (PCA) revealing dynamic but stable collective motions without major conformational changes; (F) Dynamic Cross-Correlation Matrix (DCCM) illustrating regions of correlated and anti-correlated residue motions.

(anticorrelation) blocks indicated coordinated domain movements and potential allosteric interactions. These patterns reflect structured internal communication and domain-level adaptation within CAMP1, likely in response to ligand binding (Figure 7F). The MD simulation confirmed that vaccenic acid binds stably to CAMP1, maintaining strong hydrogen bonding, compact structural conformation, and dynamic equilibrium without inducing significant conformational instability. The results underscore the therapeutic potential of vaccenic acid as a CAMP1-targeting compound and highlight the adaptability and structural integrity of CAMP1 in complex with fatty acid ligands.

A detailed 100 ns MD simulation was conducted to investigate the conformational behavior, stability, and molecular interactions of the CAMP2 protein of *C. acnes* in complex with vaccenic acid. The simulation provided

robust evidence supporting the structural integrity and dynamic compatibility of the complex, reinforcing the therapeutic relevance of vaccenic acid as a potential modulator of CAMP2 activity. The RMSD analysis revealed that the CAMP2 backbone initially exhibited minor fluctuations but stabilized around 40 ns, maintaining an average RMSD of approximately 1.3–1.4 nm for the remainder of the trajectory. This indicates that the protein achieved a stable conformation after initial equilibration. Meanwhile, vaccenic acid showed very limited conformational drift, with RMSD values consistently ranging between 0.25 and 0.35 nm, suggesting strong retention within the protein's binding pocket and a stable ligand–protein interaction throughout the simulation (Figure 8A).

Root mean square fluctuation revealed that most residues in the CAMP2 structure experienced fluctuations under 0.8 nm, with only the terminal regions showing peaks up to 1.6 nm. This pattern reflects limited local flexibility primarily at the extremities, while the core structure of CAMP2 remained rigid and stable upon ligand binding. Such structural resilience supports the idea that ligand interaction does not compromise the functional architecture of the protein (Figure 8B). The hydrogen bond profile reinforced the stability of the interaction, showing a sustained

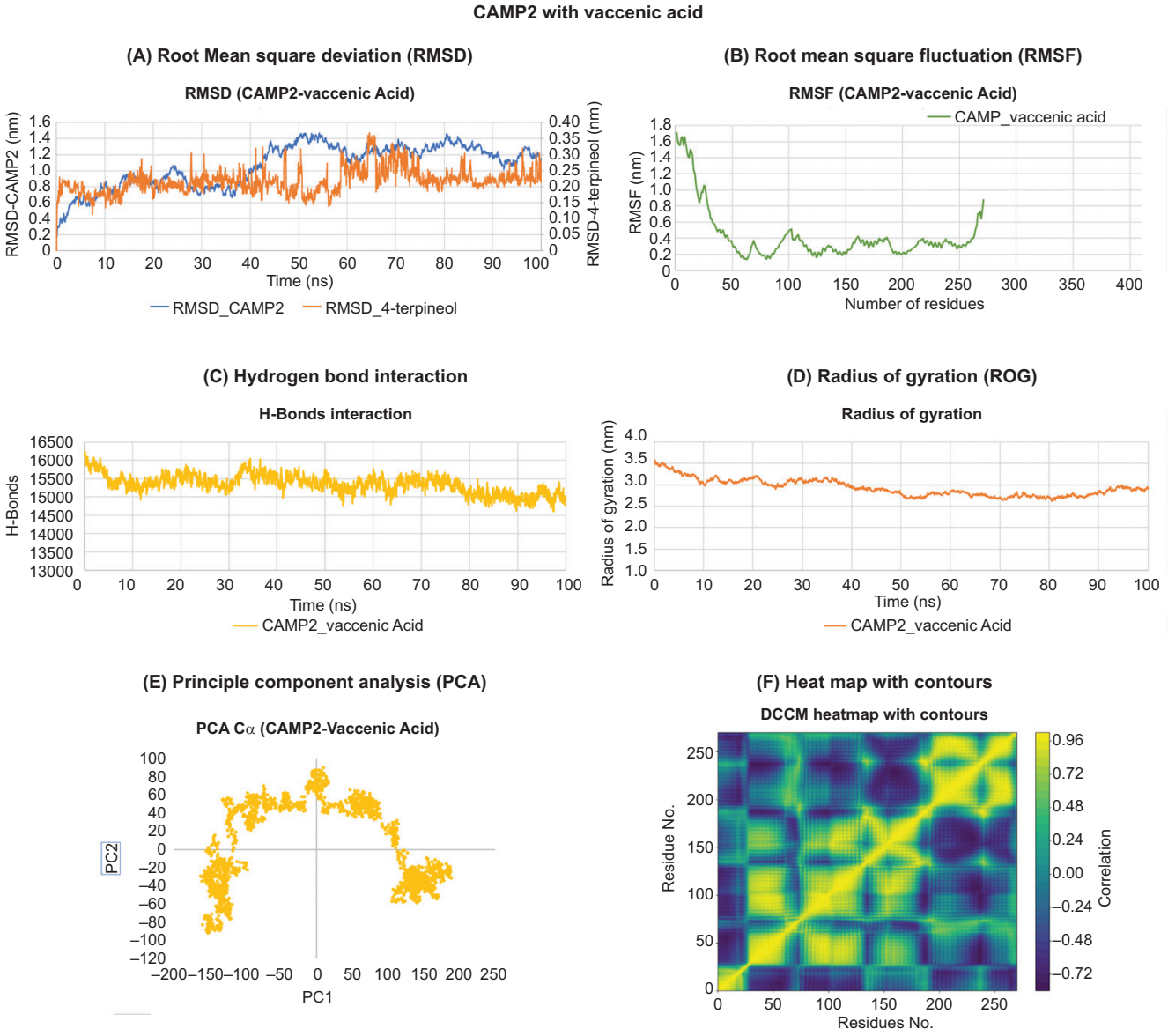

Figure 8 Molecular dynamics simulation analysis of the CAMP2–vaccenic acid complex over a 100 ns trajectory. (A) RMSD plot demonstrating the structural stability of the protein–ligand complex over time; (B) RMSF plot showing minimal residue fluctuations, indicating limited flexibility across most of the protein; (C) Hydrogen bond analysis depicting consistent intermolecular interactions between CAMP2 and vaccenic acid; (D) Radius of gyration (Rg) profile confirming the compactness and structural integrity of the complex; (E) Principal Component Analysis (PCA) illustrating stable, collective motions without significant conformational transitions; (F) Dynamic Cross-Correlation Matrix (DCCM) highlighting regions of coordinated (positively correlated) and opposing (anticorrelated) residue movements, suggesting structured internal dynamics and domain interactions.

number of hydrogen bonds fluctuating between 13,500 and 15,800 across the trajectory. This consistent bonding network indicates robust molecular recognition and energetically favorable interactions between CAMP2 and vaccenic acid, which are crucial for maintaining complex stability under physiological-like conditions (Figure 8C). In terms of compactness, the Rg plot demonstrated a slight but steady decrease over time, stabilizing around an average of 2.5 nm. This subtle compaction of the protein–ligand complex implies an adaptive tightening of the structure in response to ligand engagement, while still preserving its overall integrity and functional fold (Figure 8D). The PCA offered insight into the collective motions within the complex. The first principal component (PC1) ranged from −180 to +220, and the second (PC2) from −120 to +100, illustrating that the CAMP2–vaccenic acid complex undergoes significant yet controlled essential dynamics. The broad and scattered distribution of data points suggests flexible conformational sampling without destabilizing shifts, highlighting the natural motion and adaptability of the protein when engaged with its ligand (Figure 8E).

The DCCM revealed well-organized intra-protein motion, characterized by a dominant diagonal yellow line indicating self-correlated movements and distinct off-diagonal yellow–green and blue–purple blocks signifying positively and negatively correlated domain movements. These patterns point to orchestrated internal dynamics, where specific regions of the protein respond in a coordinated fashion to the presence of the ligand, supporting both structural adaptability and functional relevance (Figure 8F). The MD simulation of the CAMP2–vaccenic acid complex confirmed a stable, compact, and dynamically adaptive interaction, marked by sustained hydrogen bonding, limited residue fluctuation, and coordinated domain movement. These characteristics underline the potential of vaccenic acid as a stabilizing and functionally active ligand for CAMP2, offering valuable insight into its role in *C. acnes* virulence and its candidacy as a therapeutic target in acne vulgaris.

Discussion

Acne vulgaris is a chronic inflammatory skin disorder primarily driven by the activity of *C. acnes* and its virulence-associated CAMP factors. Among these, CAMP1 and CAMP2 play pivotal roles in promoting host inflammation and bacterial survival via pore-forming cytotoxic mechanisms. With the increasing prevalence of antibiotic resistance and adverse effects on the skin microbiome caused by conventional acne treatments, there is a pressing need to explore alternative therapeutic approaches. Probiotic-derived postbiotics and metabolites have emerged as promising candidates for targeted anti-acne therapies. In this context, our study employed a network pharmacology-guided strategy to identify fatty acids from *P. acidilactici* BCBH1 with the potential to inhibit CAMP1 and CAMP2 proteins of *C. acnes*. This integrated approach combined microbial metabolism data, virulence prediction, protein interaction networks, and pathway analyses to build a mechanistic foundation for probiotic-derived postbiotics in acne management.[18-20] Previous literature has documented the antimicrobial properties of LA against various skin bacteria, though its toxicity to beneficial lactic acid bacteria has limited broader applications.[21] Remarkably, *P. acidilactici* BCBH1 demonstrated exceptional tolerance to LA concentrations as high as 10% (w/v), vastly exceeding the 25 μg/mL inhibition threshold reported for other lactic acid strains. The consistent growth pattern, with a logarithmic increase in OD_{600} up to 24 h and stability through 48 h, suggests an adaptive mechanism likely involving LA detoxification via metabolic conversion to bioactive derivatives. This is supported by GC-MS findings, which revealed thirteen fatty acid metabolites produced under LA stress, notably cis-vaccenic acid peaking at 4.88 mg/L at 48 h—more than double the maximum concentrations reported in *Lactobacillus plantarum* under similar conditions.[22] The presence of long-chain fatty acids such as (Z,Z,Z)-8,11,14-eicosatrienoic acid and linoelaidic acid further exemplifies the strain's metabolic plasticity and highlights BCBH1 as a potent source of functionally diverse lipophilic compounds.

Virulence profiling of *C. acnes* CAMP proteins via the SVM-based VirulentPred algorithm confirmed their critical role in pathogenesis, with high virulence scores of 0.9055 for CAMP1 and 0.9927 for CAMP2, well above the pathogenicity threshold of 0.5. This corroborates previous findings that CAMP factors are major inducers of inflammatory cytokines such as IL-1β and TNF-α.[23] Notably, CAMP2's higher score aligns with its enzymatic function as a sialidase, implicating it in immune evasion and disruption of the skin barrier. STRING database analysis revealed moderate connectivity of CAMP1 and CAMP2 within the *C. acnes* protein interaction network (node degree of 11 each), suggesting they function in concert with other virulence and metabolic proteins rather than acting in isolation. CAMP2's GO annotations for exo-alpha-sialidase and glycosyl hydrolase activities underline its role in cleaving host glycoconjugates to facilitate bacterial persistence, consistent with reports that sialidase enzymes enhance colonization in mucosal environments.[24] Although CAMP1 lacks direct enzymatic annotations, its co-expression and clustering with CAMP2 imply a synergistic or supportive function in virulence mechanisms. Pathway enrichment analyses linked CAMP2 to key host modulation routes, including glycan degradation, sphingolipid metabolism, and innate immune system pathways—pathways well-recognized in acne-related cutaneous inflammation and lipid dysregulation.[25] The involvement of both CAMP proteins in metabolic and immune-related pathways underscores their dual role in compromising host tissue integrity while promoting bacterial survival within the pilosebaceous unit. CytoHubba analysis identified CAMP1 and CAMP2 as central hub proteins in the *C. acnes* interactome, with CAMP1 exhibiting an MCC score of 178, degree centrality of 13, and betweenness centrality of 45.27, while CAMP2 showed an MCC of 156, degree of 11, and betweenness of 26.97. These topological metrics exceed those in earlier studies and emphasize their regulatory significance in virulence and immune modulation networks. This positions CAMP1 and CAMP2 not only as virulence factors but also as critical therapeutic targets within the bacterial molecular framework.

Network pharmacology further pinpointed vaccenic acid among twelve identified metabolites as having the highest predicted binding specificity to CAMP1 (confidence score = 0.92; P = 0.04) and CAMP2 (score = 0.90; P = 0.03), substantially exceeding other metabolites (<0.7). This finding represents a significant advance over prior research,

which generally emphasized broad anti-inflammatory effects of probiotic fatty acids without elucidating direct molecular targets.[26] Molecular docking studies reinforced these observations, with vaccenic acid binding strongly to CAMP1 (binding energy of −9.6 kJ/mol) and CAMP2 (−9.3 kJ/mol), forming stable interactions with key residues such as THR68, VAL81, and ARG202 in CAMP1, and MET61, ASN75, and TYR76 in CAMP2. These binding affinities surpass those reported for natural inhibitors like palmitic acid and eugenol (−6.0 to −8.0 kJ/mol), suggesting vaccenic acid's superior potential to inhibit the pore-forming activities of CAMP proteins. For comparison, 4-terpineol, a component of tea tree oil with traditional anti-acne use, exhibited moderately strong but less favorable binding energies (−9.0 kJ/mol with CAMP1 and −8.7 kJ/mol with CAMP2) and fewer stabilizing interactions. Combined with pharmacokinetic predictions showing 4-terpineol's blood-brain barrier permeability-raising neurotoxicity concerns-vaccenic acid emerges as a safer and more effective candidate.

Toxicity and pharmacokinetic analyses revealed vaccenic acid (molecular weight 282.46 g/mol) to have high gastrointestinal absorption without BBB penetration, thereby minimizing CNS risks. Its consensus Log P of 5.7 may enhance skin permeability and retention, despite its moderate solubility (ESOL Log S = −5.41, solubility ~1.09×10^{-3} mg/mL). In contrast, 4-terpineol (154.25 g/mol) was BBB permeant with better solubility (ESOL Log S = −2.78) but potentially higher systemic absorption. Vaccenic acid's moderate bioavailability score (0.55 vs 0.85 for 4-terpineol) combined with minimal cytochrome P450 inhibition (weak inhibition only of CYP1A2 and CYP2C9) suggests a safer metabolic profile relative to 4-terpineol, which, despite lacking CYP inhibition, raises concerns due to CNS permeability. Neither compound is a P-glycoprotein substrate, supporting favorable cellular uptake without efflux-related limitations. In contrast, although 4-terpineol demonstrated favorable docking energies (binding affinities of −9.0 kJ/mol for CAMP1 and −8.7 kJ/mol for CAMP2), its pharmacokinetic liabilities and CNS penetrance limit its therapeutic scope despite its traditional use in anti-acne remedies. Molecular dynamics simulations offered further mechanistic insight, demonstrating the stable, long-term binding of vaccenic acid to CAMP1 and CAMP2. The CAMP1–vaccenic acid complex equilibrated after 40 ns, maintaining a backbone RMSD of approximately 1.4 nm and ligand RMSD of 0.2–0.25 nm, indicative of minimal conformational fluctuations and tight binding. Consistent hydrogen bonding (14,000–16,500 bonds) and a stable Rg (~2.3 nm) further confirmed the compact and robust nature of the complex. Principal component and dynamic cross-correlation analyses reflected controlled molecular motions and well-organized residue interactions, underscoring both structural stability and functional flexibility.

Similar trends were observed for CAMP2, with RMSD stabilization around 1.3–1.4 nm, ligand fluctuations between 0.25 and 0.35 nm, and hydrogen bond counts ranging from 13,500 to 15,800. The Rg averaged 2.5 nm, with slight compaction over time, indicating adaptive ligand accommodation. Broader conformational sampling without destabilization, as revealed by PCA and DCCM, suggests allosteric adjustments upon ligand binding. Together, these findings provide a comprehensive and quantitative understanding of vaccenic acid's potential to inhibit key virulence factors of *C. acnes*, surpassing prior static docking studies by elucidating dynamic stability crucial for effective protein inhibition. This supports the therapeutic promise of probiotic-derived fatty acids as novel antivirulence agents capable of mitigating acne pathogenesis while reducing antibiotic resistance risks. The combined in silico and metabolic evidence suggests vaccenic acid could serve as a safe, targeted postbiotic agent to modulate bacterial pathogenicity while preserving the skin microbiome. Future research should focus on in vitro and in vivo validation of these findings, formulation development for topical application, and exploration of synergistic effects with existing acne treatments to advance probiotic-based therapeutics for sustainable and effective acne management.

Conclusion

This study provides compelling evidence that vaccenic acid, a fatty acid metabolite derived from *P. acidilactici* BCBH1, exhibits potent inhibitory effects against the virulence-associated CAMP1 and CAMP2 proteins of *C. acnes*, key contributors to acne pathogenesis. Through a comprehensive network pharmacology approach integrated with metabolomic profiling, molecular docking, and dynamic simulation analyses, vaccenic acid demonstrated strong binding affinity, stable interaction, and favorable pharmacokinetic and toxicity profiles compared to traditional anti-acne agents such as 4-terpineol. These findings underscore the potential of probiotic-derived postbiotics as targeted, microbiome-friendly therapeutics that modulate bacterial virulence mechanisms rather than relying solely on bactericidal activity, thereby reducing the risk of antibiotic resistance and dysbiosis. Moving forward, future investigations should prioritize experimental validation using in vitro and in vivo acne models to confirm the efficacy and safety of vaccenic acid. Additionally, formulation development aimed at optimizing skin delivery and stability will be critical for clinical translation. Exploring synergistic combinations of vaccenic acid with conventional or other probiotic-derived compounds may further enhance therapeutic outcomes. Ultimately, this work opens new avenues for leveraging probiotic metabolism in the design of next-generation, sustainable acne treatments that address both microbial pathogenicity and host inflammation with precision and minimal side effects.

Availability of Data and Materials

All the data generated in this research work has been included in the manuscript.

Author's Contributions

Conceptualization: Tariq Aziz; Methodology: Jasra Naseeb; Software: Wafa A. Alshehri; Validation, Manal F. Elkhadragy and Nouf Abdullah Alharbi; Formal analysis: Abid Sarwar; Investigation: Mai M Almsaud; Resources: Zhennai Yang; Data curation: Deema Fallatah.; Writing—original draft preparation: Tariq Aziz and Jasra Naseeb; Writing—review and editing: Saleh A. Alsanie and Areej A Alhhazmi;

Visualization: Maher S Alwethaynani; Supervision: Zhennai Yang and Tariq Aziz.; Project administration: Tariq Aziz; Funding acquisition: Zhennai Yang.

Conflicts of interest

The authors declare no conflict of interest.

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