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Access Microbiology

Antimicrobial Efficacy of a Centella asiatica Extract and Its Effects on Pathogenic and Commensal Skin Bacteria

--Manuscript Draft--

Title

Antimicrobial Efficacy of a *Centella asiatica* Extract and Its Effects on Pathogenic and Commensal Skin Bacteria

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Keywords

Skin, antimicrobial management, wound healing.

Abstract

Introduction: Blastoestimulina® is a topical formulation containing *Centella asiatica* extract (10 mg/g), widely used for wound healing, scar management, and epithelial regeneration.

Gap statement: Its impact on skin microbiota remains poorly characterized.

Aim: This study aimed to evaluate the antimicrobial activity of *C. asiatica* extract and its effects on pathogenic and commensal skin bacteria.

Methodology: Antimicrobial activity was assessed in a cream (1%) formulation. The study included three phases: (1) *in vitro* suspension assays to determine antibacterial efficacy; (2) *ex vivo* human skin explant models to evaluate effects on dysbiotic skin colonized by *Staphylococcus aureus* and *Pseudomonas aeruginosa*; and (3) *ex vivo* models simulating healthy and dysbiotic skin microbiota with *S. aureus* and *S. epidermidis*. Bacterial viability was quantified by colony-forming unit (CFU) enumeration, and structural effects were visualized by scanning electron microscopy (SEM).

Results: *In vitro*, the cream exhibited broad-spectrum antimicrobial activity, significantly reducing Gram-positive (>96%, most >99.99%) and pathogenic Gram-negative (>99.99%) bacteria. *Ex vivo*, the cream reduced *S. aureus* and *P. aeruginosa* colonization under both preventive (99.96%, $p < 0.0001$; 99.00%, $p < 0.01$) and therapeutic (99.31%, $p < 0.0001$; 97.70%, $p < 0.01$) conditions. In dysbiotic models, therapeutic application selectively decreased *S. aureus* (81.18%, $p < 0.01$) while promoting growth of the key commensal *S. epidermidis* (80.18%, $p < 0.0001$);preventive application also conferred protective effects ($p < 0.05$).

Conclusion: *C. asiatica* extract effectively targets pathogenic bacteria while preserving beneficial skin microbiota, demonstrating preventive and therapeutic efficacy with potential for clinical applications in wound care and skin health management.

Introduction

The human skin is a complex and dynamic ecosystem comprising keratinocytes, immune cells, and a diverse community of bacteria, fungi, and viruses, collectively referred to as the skin microbiota. This microbial community plays an essential role in maintaining skin health by preventing colonization by pathogens, regulating immune responses, and supporting barrier integrity.[1] Dominant commensals such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Cutibacterium acnes,* and *Staphylococcus warneri* support skin barrier function and inhibit pathogen overgrowth via antimicrobial peptides (AMPs) and niche competition. However, injuries, genetic backgrounds, or external influences can disrupt this equilibrium, potentially leading to dysbiosis, which favours the expansion of opportunistic pathogens.[2,3]

ESKAPE pathogens—an acronym for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species—represents a group of opportunistic bacteria notorious for their ability to "escape" the effects of antimicrobial agents and evade host immune defenses. These pathogens are significant contributors to antibiotic resistance and are commonly involved in skin and soft tissue infections, particularly in wounds.[4–7]

The global rise in antimicrobial resistance among ESKAPE pathogens underscores the urgent need for alternative strategies that both supress harmful bacteria and preserve beneficial skin microbiota. [8,9] In line with recommendations from European health authorities,[10] which promote the development of non-traditional approaches to limit resistance, plant-derived compounds in topical formulations represent a promising, regulation-aligned strategy. Bioactive plant compounds have emerged as hopeful candidates due to their broad antimicrobial, anti-inflammatory, and barrier-restoring properties.[8,9] These molecules, such as the triterpenes, offer advantages over traditional antibiotics by acting via multiple mechanisms, reducing the risk of resistance, and exhibiting a favorable safety profile in topical applications.[11,12] *Centella asiatica*, a medicinal plant long used for wound healing and skin regeneration, contains key triterpenoids such as asiaticoside, madecassoside, asiatic acid, and madecassic acid. These compounds promote collagen synthesis, angiogenesis, and keratinocyte migration, while also providing antioxidant and antimicrobial effects.[13–15]

Blastoestimulina® is a topical formulation containing *C. asiatica* extract (10 mg/g), commercially available as a cream or powder. It has been widely used in clinical practice for wound healing, surgical scar management, and epithelial regeneration; however, its potential impact on the skin microbiota has not been thoroughly characterised.[16] To adress this gap, a preclinical study employing *in vitro* and *ex vivo* human skin explant models was conducted to assess the antimicrobial and microbiome-modulating properties of Blastoestimulina®. The study aimed to determine whether *C.*

asiatica–based treatment can simultaneously inhibit pathogenic bacteria and preserve commensal species, thereby contributing to the maintenance of skin homeostasis and supporting wound healing. Beyond the bioactive properties of *C. asiatica*, the investigation also assessed the contribution of the cream's physical characteristics, including its filmogenic, preventive barrier, to its overall antimicrobial protection.

Methods

Study Design Overview

The study was conducted in three experimental phases to evaluate the antimicrobial, microbiome-modulating, and formulation-related protective properties of the *C. asiatica*–based cream (Blastoestimulina®) using complementary *in vitro* and *ex vivo* approaches. To provide a comprehensive visual summary of the experimental design, the sequential workflow of the study is depicted in *Figure 1*.

- **Phase 1: *In Vitro* Suspension Assay**

Bacterial suspensions were prepared from selected pathogenic (*S. aureus*, *P. aeruginosa, K. pneumoniae, A. baumannii, E. faecalis,* and *E. aerogenes*) and commensal (*S. epidermidis, S. hominis,* and *S. warneri*) strains. 1 g of Blastoestimulina® (cream) was incubated with each suspension for 24 hours. Viable cells were quantified by serial dilution and plate counting, and the antimicrobial activity was expressed as the percentage reduction in colony-forming units (CFU) compared with untreated controls.

- **Phase 2: *Ex vivo* Human Skin Explant Model**

Human skin explants from healthy donors were cultured under controlled conditions. Dysbiotic skin states were induced by inoculating explants with *S. aureus* or *P. aeruginosa*. Blastoestimulina® cream was applied either preventively (pre-inoculation) or therapeutically (post-colonization). Treatment efficacy was evaluated by CFU enumeration and scanning electron microscopy (SEM) to assess bacterial biofilm formation and skin surface morphology.

- **Phase 3: *Ex* vivo Skin Explant Model with Mixed Bacterial Populations**

Skin explants were inoculated with mixed bacterial suspensions to simulate either a dysbiotic skin balance (*S. aureus* : *S. epidermidis*, 1:1) or a healthy balance (1:10). Blastoestimulina® cream was applied either as a preventive measure or as a therapeutic treatment. Bacterial viability was determined by CFU counting, and species differentiation was based on colony morphology observed on agar plates.

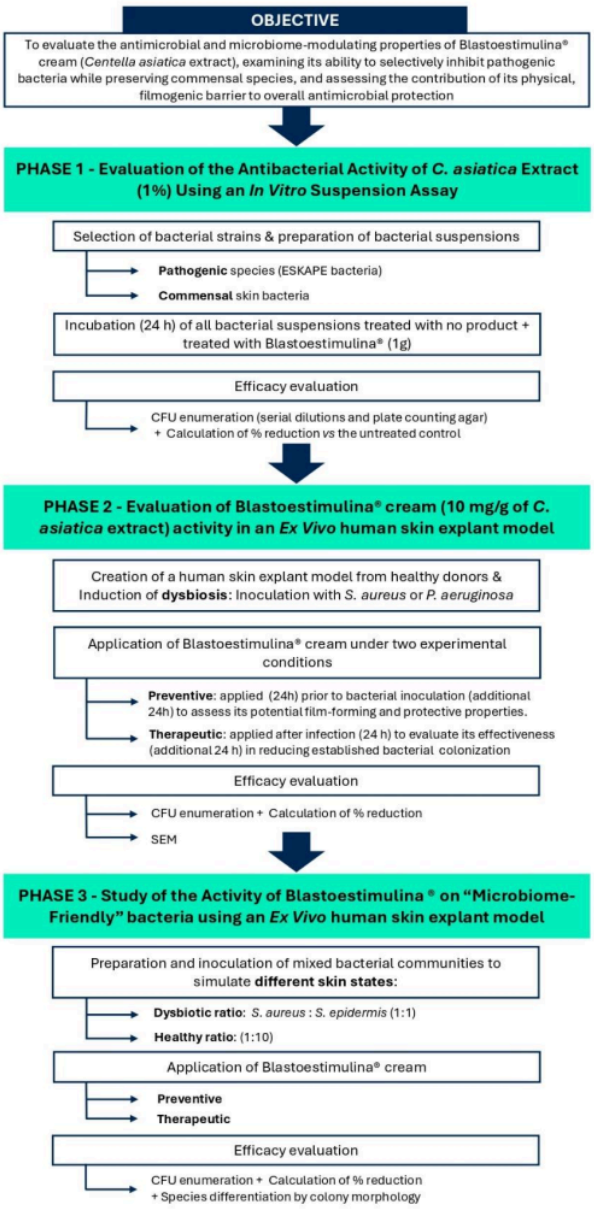

Figure 1. Overview of the experimental workflow. Abbreviations: CFU, colony-forming units; SEM, scanning electron microscopy. In phase 1, ESKAPE pathogens correspond to *S. aureus*, *P. aeruginosa, K. pneumoniae, A. baumannii, E. faecalis,* and *E. aerogenes*; while commensal bacteria to *S. epidermidis, S. hominis,* and *S. warneri* strains.

Materials, Equipment, and Test Product

Experiments were conducted using a range of microbiological media (Nutrient Agar [NA], PBS, Maximum Recovery Diluent [MRD], Ringer solution) and hOSECs obtained from healthy donors. Laboratory equipment—including laminar flow cabinets, CO_2 incubators, automatic colony counters, and SEM—was calibrated and operated following ISO and GLP standards.

The test product, Blastoestimulina® (Almirall, S.A.) containing *C. asiatica* extract, was supplied in a cream (1%) formulation (*Figure 2*). For *in vitro* suspension assays, 1 g of Blastoestimulina® was incubated with bacterial suspensions for 24 hours. For *ex vivo* skin experiments, the cream was applied topically over skin explant surface using a micropipette. Subsequently, product was spread on the surface.

Control groups were included for all experiments: bacterial suspensions or skin explants without Blastoestimulina® (0% extract) were processed in parallel to quantify baseline microbial growth and ensure the observed effects were attributable to the test product.

Bacterial Strains and Culture Conditions

Pathogenic and commensal bacterial strains were obtained from the Spanish Type Culture Collection (CECT) or clinical isolates. Pathogens included *S. aureus* (CECT 239), *P. aeruginosa* (CECT 116), *K. pneumoniae* (clinical isolate), *A. baumannii* (clinical isolate), *E. faecalis* (CECT 184), and *E. aerogenes* (CECT 684). Commensal species included *S. epidermidis* (CECT 231), *S. hominis* (CECT 234), and *S. warneri* (CECT 236).

All strains were cultured on NA at 37°C for 24 h under aerobic conditions. Working bacterial suspensions were standardized per each experimental phase. For *in vitro* assays (Phase 1), suspensions were adjusted to 1–5 × 10^5 CFU/mL per strain. For *ex vivo* assays (Phases 2 and 3), 20 µL aliquots of bacterial suspensions were applied to the surface of human skin explants. Phase 2 employed individual *S. aureus* or *P. aeruginosa* inoculations to model dysbiotic skin conditions. Phase 3 used mixed *S. aureus* and *S. epidermidis* inocula at healthy (1:10; 10^6 CFU/explant *S. aureus* and 10^7 CFU/explant *S. epidermidis*) or dysbiotic (1:1; 10^6 CFU/explant each) ratios to mimic skin microbiota.

Ex Vivo Human Skin Explant Model

hOSECs were obtained with informed consent from healthy donors undergoing plastic surgery. Donor samples were fully anonymized prior to receipt by the investigators. Skin was processed within

2 hours post-surgery into 0.8 cm² pieces and maintained in transport medium. Explants were cultured dermis-side down in plates containing serum-free skin culture medium supplemented with 1% penicillin-streptomycin and incubated at 37°C under 5% CO_2 for 48 hours to allow recovery before experimentation.

For the *ex vivo* phase evaluating *S. aureus* and *P. aeruginosa* (Phase 2), a total of 20 explants were used (four explants for CFU quantification and one explant for SEM analysis per experimental group). All explants in this phase were obtained from a single donor (Caucasian female, 29 years old).

For the *ex vivo* phase involving mixed bacterial populations (*S. aureus* and *S. epidermidis*, Phase 3), 24 explants were used (four explants for CFU quantification and one explant for SEM analysis per experimental group). These explants were obtained from a different single donor (Caucasian female, 47 years old).

Antimicrobial Activity Assays

Phase 1 – *In vitro* suspension assay

The first phase of the study was designed to evaluate the direct antimicrobial activity of Blastoestimulina® against different bacterial strains under controlled laboratory conditions.

Bacterial suspensions were incubated for 24 h with Blastoestimulina® (cream, 1 g per assay). Viable bacteria were quantified by serial dilution and plate counting on NA. Results were expressed as percentage reduction in viable cells compared with untreated controls, using the formula:

$$\text{Reduction (\%)} = \frac{A - B}{A} \times 100$$

Where A represents mean CFU in control suspensions and B CFU after product exposure. Each assay was performed in triplicate.

Phase 2 & 3 – *Ex vivo* human skin explant model & *ex vivo* human model with mixed bacterial populations

In the second phase, an *ex vivo* human skin explant model was employed to evaluate the product's efficacy in a more physiologically relevant context, simulating both healthy and dysbiotic conditions. Subsequently, the third phase expanded this assessment by investigating Blastoestimulina® effects on

pathogen-commensal interactions in skin explants inoculated with mixed bacterial suspensions that simulate healthy and dysbiotic microbiota.

Following treatment and incubation, the surface of each skin explant was gently washed with phosphate-buffered saline to remove non-adherent bacteria. Samples were then processed to quantify viable cells by serial dilution and plating on NA. Aliquots (100 µL) from each dilution were seeded and incubated at 37°C for 24 h under aerobic conditions. CFU were enumerated and expressed as CFU/cm² of skin surface to evaluate the antimicrobial efficacy of Blastoestimulina® cream under preventive and therapeutic conditions. Each experimental group was analysed in quadruplicate. A summary of the experimental design, including treatment conditions, bacterial conditions and replicate numbers, is presented in the *Supplementary Table 1*.

Scanning Electron Microscopy (SEM)

For morphological assessment, one replicate per group was processed for SEM. Samples were fixed in 2% glutaraldehyde, washed in phosphate buffer, dehydrated through graded ethanol (30–100%), and air-dried after hexamethyldisilazane treatment. Dried samples were gold-coated and imaged under a scanning electron microscope to visualize bacterial adherence and biofilm formation.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Differences between treated and control groups were assessed using unpaired *t*-test. A *p*-value ≤ 0.05 was considered statistically significant.

Results

Phase 1: *In Vitro* Suspension Assay

Activity against ESKAPE and Opportunistic Pathogens

Among Gram-positive strains, *S. aureus* was highly susceptible to Blastoestimulina® cream, showing a >99.99% reduction in CFUs ($p < 0.001$) (*Table 1*, *Figures S1–S2*). In contrast, *E. faecium* showed limited sensitivity, with a non-significant CFU decrease of 42.62%.

For Gram-negative pathogens, the cream demonstrated broad-spectrum bactericidal activity, with CFU reductions exceeding 99.99% for *A. baumannii* ($p < 0.05$), *K. pneumoniae* ($p < 0.0001$), *P. aeruginosa* ($p < 0.01$), and *E. faecalis* ($p < 0.01$).

Activity against Commensal Skin Bacteria

Blastoestimulina® also affected commensal *Staphylococcus* species dominant in healthy skin microbiota, although to a lesser extent than pathogenic strains. *S. epidermidis* exhibited a moderate but significant reduction of 96.82% ($p < 0.0001$). *S. hominis* was highly sensitive, with reductions exceeding 99.99% ($p < 0.01$). For *S. warneri*, the cream achieved strong inhibition of 99.99% ($p < 0.0001$).

Table 1. Summary of percentage increases (↑) or decreases (↓) in bacterial viability and corresponding log_{10} reductions after 24-hour exposure of Blastoestumilina®. *, p<0.05; **, p<0.01; ***, 0.001; ****, p<0.0001; n.s, non-significant.

Bacteria	***Centella asiatica* 1% (24h)** Blastoestimulina® cream	
	CFU % reduction	**Log_{10} reduction**
Staphylococcus aureus	>99.99(↓)***	-5.80
Pseudomonas aeruginosa	>99.99(↓)**	-4.68
Klebsiella pneumoniae	>99.99(↓)****	-7.44
Enterobacter aerogenes	>99.99(↓)**	-7.09
Acinetobacter baumannii	>99.99(↓)*	-5.54
Enterococcus faecalis	42.62(↓) (n.s)	-0.24
Staphylococcus epidermidis	96.82(↓)****	-1.50
Staphylococcus hominis	>99.99(↓)**	-5.74
Staphylococcus warneri	99.99(↓)****	-4.11

Phase 2: *Ex vivo* Human Skin Explant Model

Preventive regimen

When applied prior to bacterial inoculation, the cream exhibited pronounced bactericidal activity, achieving >99.96% CFU reduction for *S. aureus* ($p < 0.0001$) and >99.00% reduction for *P. aeruginosa* ($p < 0.01$) (*Figure 3*). These findings indicate that topical application of Blastoestimulina® provides a protective barrier for the skin, preventing bacterial adhesion and early colonization on its surface.

Therapeutic regimen

Under therapeutic conditions, where treatment was applied following bacterial infection, Blastoestimulina® cream showed significant antibacterial efficacy, reducing *S. aureus* by over 99.31% ($p < 0.0001$) and *P. aeruginosa* by more than 97.70% ($p < 0.01$). These data demonstrate that the formulation remains effective even after pathogen establishment.

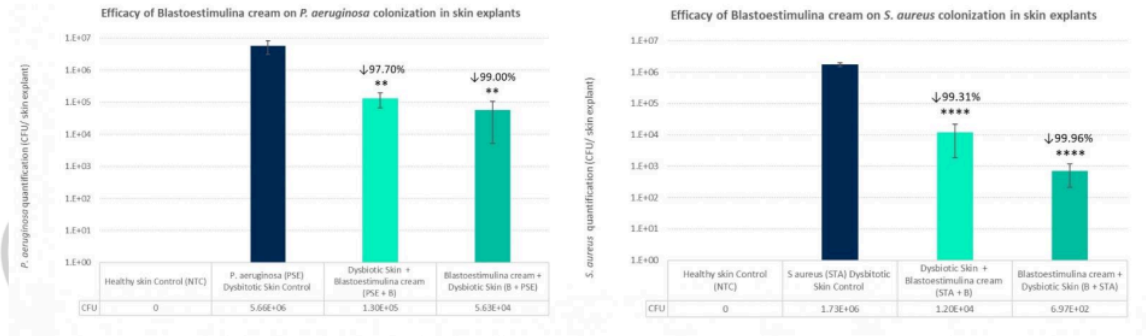

Figure 2. Mean CFU values of *S. aureus* and *P. aeruginosa* recovered from human skin explants following treatment with Blastoestimulina® cream. **, p<0.01; ****, p<0.0001. Abbreviations: PSE, *P. aeruginosa;* STA, *S. aureus*; B, Blastoestimulina® cream. "STA or PSE + B" indicates therapeutic treatment (application after bacterial inoculation), whereas "B + STA or PSE" denotes preventive treatment (application prior to bacterial inoculation).

SEM analysis

Complementary SEM analysis on human skin explants corroborated the trends observed in the CFU quantification of *S. aureus* and *P. aeruginosa* (*Figure 4*). In treated skin explants, scattered or absent bacterial cells were observed. Additionally, no bacterial colonization was visible when the cream was applied as a filmogenic preventive barrier, indicating strong physical and antimicrobial protection.

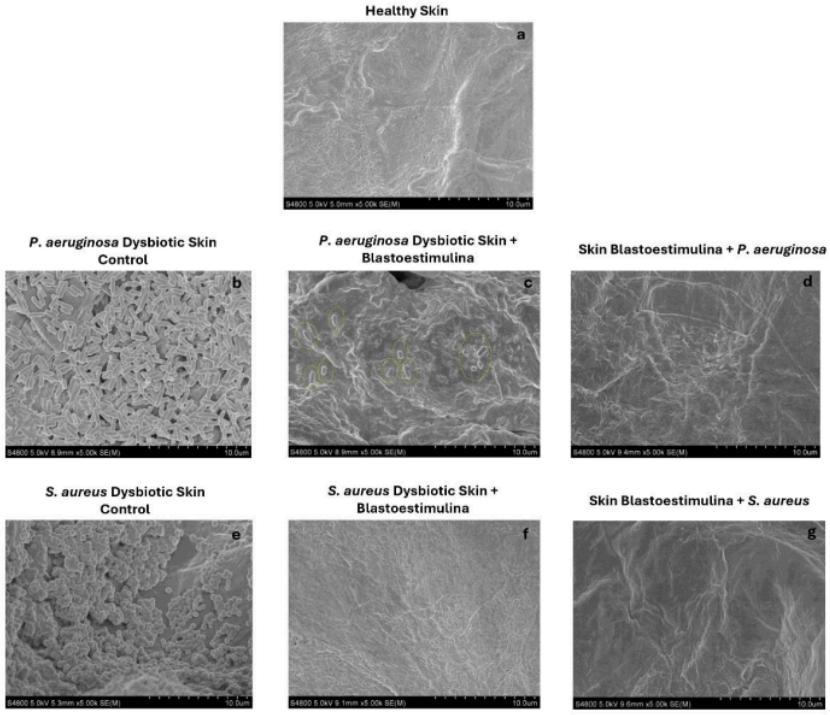

Figure 3. SEM images x5000 of human skin explants samples verifying the trends observed in bacterial CFU quantification. (a) Healthy skin (NTC) samples. No bacteria + No Test product human skin; (b) *P. aeruginosa* Dysbiotic Skin Control (No Test product) skin samples; (c) *P aeruginosa* + Test product (applied as a treatment) skin samples. Some residual bacteria (marked as yellow circles) are seen on skin surface; (d) Test product (applied as preventive filmogenic barrier) + *P. aeruginosa* skin samples; (e) *S. aureus* Dysbiotic Skin Control (No Test product) skin samples; (f) *S. aureus* + Test product (applied as a treatment) skin samples; (g) Test product (applied as preventive filmogenic barrier) + *S. aureus* skin samples.

Phase 3: *Ex vivo* Skin Explant Model with Mixed Bacterial Populations

Healthy microbiota conditions

Under conditions simulating a balanced skin microbiota, application of Blastoestimulina® cream led to a significant reduction of *S. aureus* by 90.78% ($p < 0.05$). In contrast, *S. epidermidis* exhibited a non-significant increase of 34.9% (*Figure 5*). These results indicate a selective inhibitory effect on pathogenic *S. aureus* while preserving the commensal *S. epidermidis* population.

Therapeutic regimen under dysbiosis

Following the induction of dysbiosis dominated by *S. aureus*, therapeutic application of Blastoestimulina® achieved an 81.18% reduction of *S. aureus* ($p < 0.01$). Notably, *S. epidermidis* increased substantially by 80.18% ($p < 0.0001$), thereby restoring a more favourable commensal-to-pathogen ratio.

Preventive regimen under dysbiosis

Pre-application of the cream prior to bacterial inoculation reduced *S. aureus* by 64.12% ($p < 0.05$), while *S. epidermidis* showed a non-significant increase by 16.71%. These findings suggest that Blastoestimulina® can act both as a preventive barrier and a therapeutic modulator, selectively targeting pathogens while promoting commensal survival.

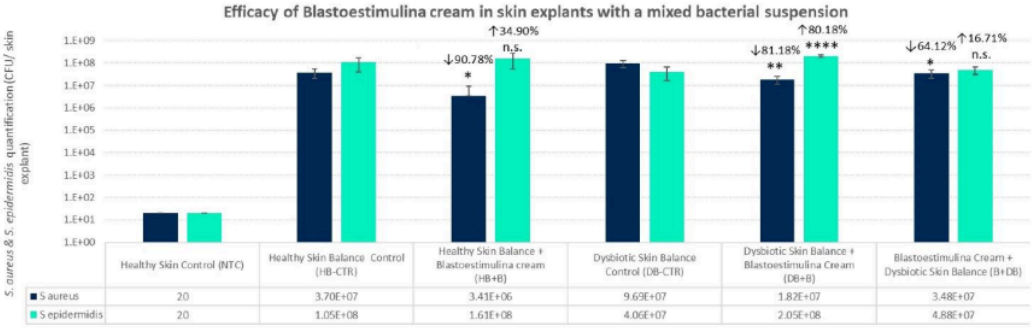

Figure 4. Mean CFU values of *S. aureus* and *S. epidermidis* measured in mixed bacterial suspension study following treatment with the tested product. When no CFUs were detected, a value of 20 CFU was assigned, corresponding to the detection limit of the method. Measurements were performed at 48 h. *, p<0.05; **, p<0.01; ****, p<0.0001; n.s, non-significant. Abbreviations: B, Blastoestimulina® cream; HB, Healthy Skin Balance; DB, Dysbiotic Balance. "DB or HB + B" denotes therapeutic treatment (product applied after bacterial inoculation), whereas "B + DB" denotes preventive treatment (product applied prior to inoculation).

Discussion

This study demonstrates that Blastoestimulina® cream, containing *C. asiatica* extract, exhibits selective and potent antimicrobial activity against skin-associated bacteria. The 1% cream effectively inhibited major ESKAPE pathogens such as *S. aureus* and *P. aeruginosa*, which are frequently implicated in skin and wound infections.[1,7] In contrast, the treatment preserved or even enhanced the growth of commensal species such as *S. epidermidis* and *S. hominis*, microorganisms that play essential roles in maintaining skin homeostasis.[3] This selective activity suggests that Blastoestimulina® functions

not only as a bactericidal agent but also as a microbiome-modulating therapy capable of restoring the skin's natural microbial balance. These results are consistent with emerging evidence showing that *C. asiatica*–derived probiotics promote beneficial microbial populations while limiting pathogenic overgrowth.[17] Importantly, these findings extend the therapeutic relevance of Blastoestimulina®, indicating its potential to prevent and manage dysbiosis-associated complications during wound healing.

The observed antimicrobial efficacy aligns with the well-characterized bioactive triterpenoids of *C. asiatica* (asiaticoside, madecassoside, and asiatic acid), which exert antimicrobial, antioxidant, and anti-inflammatory effects.[15,18–20] These compounds can disrupt bacterial membranes, inhibit biofilm formation, and mitigate oxidative stress in injured tissue.[21] Previous investigations have reported strong inhibition of *S. aureus*, methicillin-resistant *S. aureus*, and *P. aeruginosa* following exposure to *C. asiatica* extracts or nanoparticle-based formulations,[22,23] further corroborating the present results. Interestingly, similar species-specific effects have been described for other plant-derived phenolic compounds, such as those from pomegranate peel extract, which reduced *S. aureus* biofilm formation while promoting *S. epidermidis* growth in mono- and dual-species biofilm models.[24] This suggests that some natural extracts may share a microbiota-modulating mechanism, selectively inhibit pathogenic *staphylococci* while supporting the beneficial commensals that contribute to cutaneous homeostasis.

Beyond its intrinsic antimicrobial activity, the galenic properties of the formulation also contribute to the overall protective effect of Blastoestimulina®. As a topical cream, its physical vehicle forms a persistent, filmogenic barrier over the skin surface, limiting the penetration of external contaminants and reducing the risk of colonization by new pathogens.[25] Additionally, the formulation helps maintain a localized and sustained concentration of the bioactive compounds at the site of application, thereby enhancing their antimicrobial performance. In wounds and burns, where microcirculation may be severely impaired, rendering systemic antibiotics less effective, topical antimicrobials strategies become particularly important. Furthermore, by suppressing microbial growth directly at the injured site, the formulation establishes a microenvironment that favours cutaneous cell activity, facilitating tissue repair and recovery.[26] Collectively, these characteristics indicate that the formulation acts not only as a carrier of active triterpenoids but also as a protective barrier system that reinforces the preventive and therapeutic potential of the product.

The *ex vivo* human skin explant model offers a physiologically relevant platform to assess antimicrobial activity, as it maintains the structural integrity and cellular composition of human skin.[27,28] Within this system, Blastoestimulina® cream demonstrated efficacy under both preventive and therapeutic conditions, markedly reducing pathogenic colonization while maintaining tissue integrity. These results reinforce that the formulation functions as a barrier-protective and reparative agent, promoting a microenvironment conducive to beneficial microbiota. This activity is consistent with the

well-established wound-healing, anti-inflammatory, and antimicrobial properties of *C. asiatica*, which promotes fibroblast proliferation, collagen synthesis, angiogenesis, and epithelialization.[13,29] The concurrent regenerative and microbiome-supportive effects are particularly valuable in dysbiotic or damaged skin, where microbial imbalance delays repair.[28] Preservation of commensal species such as *S. epidermidis* further supports the microbiome-friendly profile of Blastoestimulina®, as these organisms enhance host defence through antimicrobial peptide production and modulation of immune and regenerative responses.[27,30] Collectively, these mechanisms contribute to accelerated healing, reduced inflammation, and restoration of skin homeostasis.[30]

In conclusion, Blastoestimulina® cream showed potent and selective antimicrobial activity against key skin pathogens while preserving beneficial commensal species, underscoring its microbiome-friendly profile. Its galenic structure further enhances protection by creating a filmogenic barrier that limits pathogen adhesion and supports tissue repair. These combined effects make Blastoestimulina® a valuable tool for wound care, chronic or recurrent skin infections, postoperative management, and the prevention of dysbiosis-related complications. Moreover, its ability to control pathogens while preserving the resident microbiota aligns with European public health priorities that promote non-antibiotic alternatives. This positions the product as a clinically relevant and sustainable approach to reducing antimicrobial resistance.

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This study presents several methodological and conceptual strengths. The combined use of *in vitro* suspension assays and *ex vivo* human skin explant models provided complementary insights into the antimicrobial and microbiome-modulating effects of Blastoestimulina®. Inclusion of both pathogenic and commensal bacteria enabled assessment of selective antimicrobial activity, while preventive and therapeutic testing revealed its dual potential for infection control and microbiota restoration. Conducting the human skin explant experiments ensured reproducibility and physiological relevance, and the defined dosing protocol supported comparability across phases. Together, these features enhance the reliability and clinical applicability of the findings.

Despite its robust experimental design, this study has certain limitations that need consideration. The *ex vivo* skin model, lacks systemic physiological components such as vascularization, immune cell infiltration, and long-term microbiome dynamics, which are crucial for replicating *in vivo* host–microbe interactions. Additionally, only a single product concentration and formulation type were tested, precluding assessment of dose-dependent effects or vehicle influence on bioavailability. The relatively short observation period (24–48 hours) may not fully capture delayed antimicrobial or regenerative responses. Moreover, the absence of molecular or biochemical assays to elucidate mechanisms of action limits mechanistic interpretation.

Future research should validate these findings through *in vivo* clinical studies to confirm the efficacy and microbiome-modulating effects of Blastoestimulina®. Molecular approaches such as transcriptomic, metabolomic, and immunological analyses are needed to clarify its mechanisms of action. *Ex vivo* and *in vivo* evaluation of the powder formulation (20 mg/g) will also be important to determine whether its higher concentration enhances antimicrobial and regenerative effects, as suggested by related evidence on dose-dependent bioactivity of *C. asiatica* constituents.[13,20]

Author contributions

DA and ND participated in the approval, initiation, design, and analysis of the entire research; AA performed the experiments; all authors reviewed and approved the final version of the manuscript.

All authors have reviewed and approved this transparency declaration and take responsibility for its content.

Conflicts of interest

DA and ND are employees of ALMIRALL S.A., the funder of this research. DA holds shares in ALMIRALL S.A. All other authors: none to declare. The funder has not played any role in the design, execution, analysis, or writing of the study, beyond providing funding and material resources. No professional medical writing assistance or similar services were received, except for the support of Angel Ramírez and Kangkana Banerjee from Alta Medical Services for medical writing, as acknowledged in the Acknowledgements section. No author has received additional reimbursement for preparing the article.

Ethical approval

Human organotypic skin explant cultures (hOSECs) were commercially obtained from Biopredic International, a certified and accredited tissue provider operating under established ethical and regulatory frameworks governing the procurement and distribution of human tissues exclusively for research purposes. Donors provided documented informed consent prior to tissue collection.

Tissue procurement was authorized by the competent French governmental ethics authority in accordance with French law (Code de la Santé Publique, Article L.1245) and complies with applicable European regulations governing human tissues and cells. The supplier holds all required licenses and

regulatory authorizations for the collection, processing, international transfer, and export of human tissues for research use.

Samples were fully anonymized prior to receipt by the investigators, and no identifiable personal data were accessible at any stage of the study, in compliance with the General Data Protection Regulation (GDPR) and relevant European data protection legislation. As no human participants were recruited and no identifiable data were handled by the research team, additional institutional ethical review was not required under regulations governing the secondary use of anonymized human biological materials, in accordance with Spanish Law 14/2007 on Biomedical Research.

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Supplementary Materials

Table S1. Summary of Experimental Design and Evaluation of the Study.

B®: *Blastoestimulina®*; CFU: *Colony Forming Units*; hOSEC: *Human Organotypic Skin Explant Culture*; NA: *Nutrient Agar*; SEM: *Scanning Electron Microscopy*.

Phase	Group	Group Name	Description / Treatment Protocol	Bacterial Condition	Treatment Timing	Evaluation Method (and Replicates)
I	1	Bacterial Suspension Control	Bacterial suspension treated with no product	ESKAPE pathogens & healthy microbiota	—	CFU (3)
	2	Bacterial suspension + B®	Bacterial suspension treated with B® (1 g)	ESKAPE pathogens & healthy microbiota	Pre-infection	CFU (3)
II	1	Healthy Skin Control (NTC)	Untreated hOSEC, no bacteria, no product.	—	—	CFU (4) + SEM (1)
	2	Dysbiotic Skin Control	hOSEC inoculated with *S. aureus* or *P. aeruginosa*. No product applied.	Pathogenic (dysbiotic)	—	CFU (4) + SEM (1)
	3	Dysbiotic Skin + B®	hOSEC first inoculated with pathogens, then treated with B® cream (2 mg/cm²).	Pathogenic	Post-infection (therapeutic)	CFU (4) + SEM (1)
	4	B® + Dysbiotic Skin	hOSEC pre-treated with B® cream (2 mg/cm²), then infected with *S. aureus* or *P. aeruginosa*.	Pathogenic	Pre-infection (preventive)	CFU (4) + SEM (1)
III	1	Healthy Skin Control (NTC)	Untreated hOSEC, no bacteria, no product.	—	—	CFU (4)
	2	Healthy Balance + No Product	hOSEC inoculated with healthy bacterial ratio (1:10; *S. aureus: S. epidermidis*).	Healthy microbiota	—	CFU (4)
	3	Healthy Balance+ B®	hOSEC inoculated with healthy ratio (1:10), then treated with B® cream.	Healthy microbiota	Post-infection	CFU (4)
	4	Dysbiotic Balance + No Product	hOSEC inoculated with dysbiotic bacterial ratio (1:1; *S. aureus*: *S. epidermidis*).	Dysbiotic microbiota	—	CFU (4)
	5	Dysbiotic Balance+ B®	Dysbiotic ratio inoculation, followed by B® treatment.	Dysbiotic microbiota	Post-infection	CFU (4)
	6	B® + Dysbiotic Balance	hOSEC pre-treated with B® cream, then inoculated with dysbiotic ratio (1:1).	Dysbiotic microbiota	Pre-infection	CFU (4)

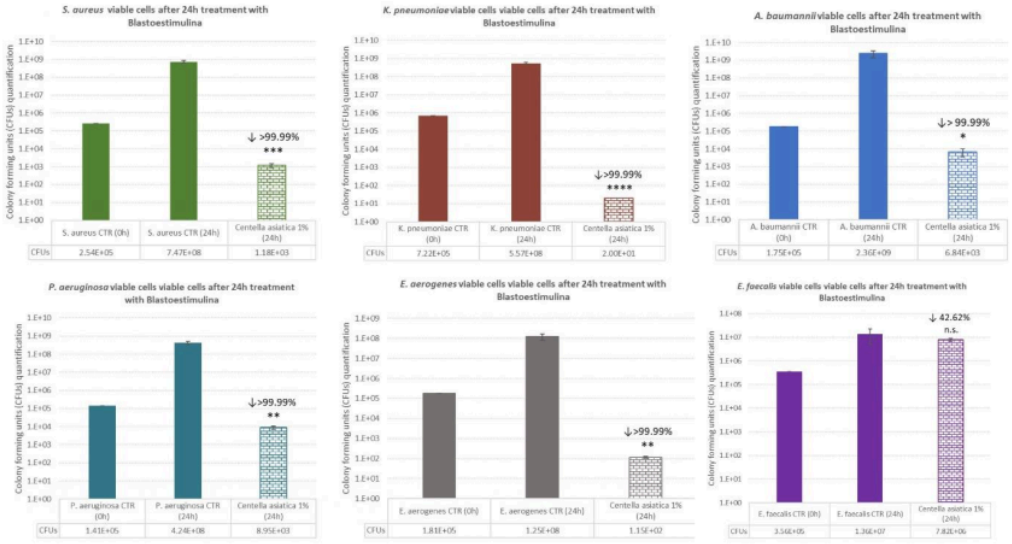

Figure S1. Mean CFU values of ESKAPE pathogens after 24-hour treatment with Blastoestimulina® cream in suspension assay. *, p<0.05; **, p<0.01; ***, 0.001; ****, p<0.0001; n.s, non-significant.

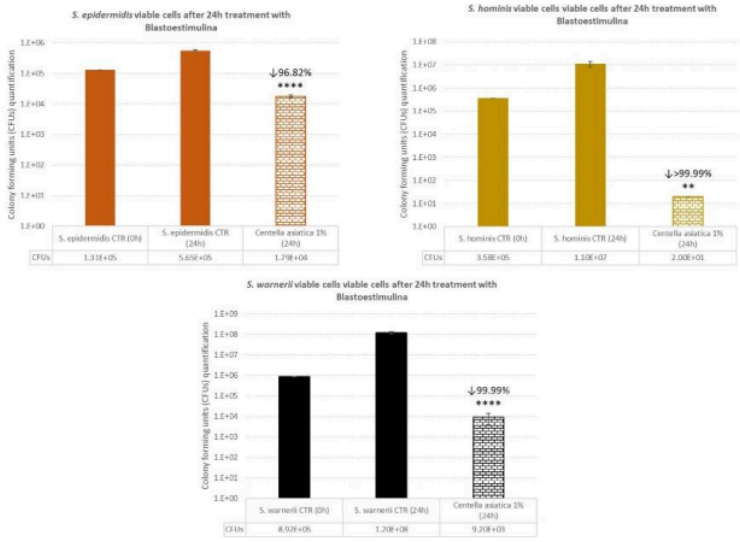

Figure S2. Mean CFU values of commensal skin bacteria after 24-hour treatment with Blastoestimulina® cream in suspension assay. **, p<0.01; ****, p<0.0001.

Raw data

Raw data

Raw data

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