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Novel Topical Application of a Postbiotic, LactoSporin®, in Mild to Moderate Acne: A Randomized, Comparative Clinical Study to Evaluate its Efficacy, Tolerability and Safety †

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Abstract: Acne vulgaris is a common skin disorder of pilosebaceous units. The therapy for mild-to-moderate acne includes topical antibiotics, benzoyl peroxide and retinoids. In this open-label, randomized monocentric study, we compared the efficacy of LactoSporin® 2% *w/w* cream with benzoyl peroxide in 64 male and female subjects with mild-to-moderate acne for three weeks. The efficacy parameters were evaluated based on the dermatologist visual assessment and instrumental measurements using Sebumeter® MPA580, Antera 3D™ and VISIA CR 2.2 and subject self-assessment questionnaires. Adverse events were recorded throughout the study period. In order to understand the mechanism of action and properties of LactoSporin, the pH stability, thermostability, antimicrobial activity and 5-alpha reductase activity were evaluated in vitro. A significant improvement was observed in the dermatological assessment of closed comedones ($p < 0.0001$), open comedones ($p = 0.0069$) and papules count ($p < 0.0001$) in comparison to the baseline in both LactoSporin and benzoyl peroxide groups. The antera analysis showed significant improvement in redness ($p < 0.0001$) and elevation ($p < 0.0001$) (small and medium) in both the treatment groups. The sebumeter analysis showed a significant decrease in sebaceous secretion ($p < 0.0001$) for LactoSporin, which resulted in reduced oiliness, pimples, acne spots and redness around the acne spot. The product was found to be safe without any irritancy. LactoSporin was stable at an acidic pH and temperature range of 70 to 90 °C, with antimicrobial activity against various pathogenic bacteria, including *Cutibacterium acnes*. It was also a potent inhibitor of 5-alpha reductase activity. Thus, it can be concluded that the efficacy of LactoSporin is equivalent to benzoyl peroxide in the treatment of mild-to-moderate acne lesions and better than benzoyl peroxide for reducing the sebaceous secretion and oily, greasy nature of the skin, implying its efficacy in other sebohorriec conditions.

Keywords: primary skin irritation patch test; antiacne; postbiotics; LactoSporin®; benzoyl peroxide

1. Introduction

Acne vulgaris is a common skin disorder of the pilosebaceous unit present in the face, neck, chest and back that usually affects all individuals once in their life but usually peaks at puberty in both males and females [1,2]. It is the eighth most prevalent disease worldwide, accounting for 9.4% of the

global population, of which 85% are young adults [3]. Acne is characterized by comedones, papules, pustules, cysts, nodules and often scars in the affected area [4].

The pathogenesis of acne vulgaris is still unclear, but various reasons known so far are the obstruction of sebaceous follicles, hyper-keratinization, androgen-induced excessive sebum production, inflammation and bacterial colonization of hair follicles by *Cutibacterium acnes* [5,6]. Often, the painful inflammatory lesions on the skin drive individuals to seek treatment. Though acne is not a life-threatening disease, the developed scares often leads to psychological effects like social isolation, depression, anxiety and suicidal ideation, increasing the disease burden [3,7,8]. Thus, in order to minimize these impacts, therapy based on etiopathological factors, such as anti-comedogenic, anti-inflammatory, antimicrobial agents and hormonal therapy, are in practice [9]. Among the available treatments, retinoids remain the mainstay topical therapy due to their comedolytic effect, microcomedone-resolving activity, anti-inflammatory activity and acne clearance [10]. Topical benzoyl peroxide is used as the first line of treatment in some countries, as per some medical guidelines. Benzoyl peroxide has antibacterial activity against *C. acnes*, but its high oxidative potential bleaches hair, skin and even clothing. It is also reported to have an excessive peeling effect, redness of the skin, difficulty in breathing, skin irritation and skin dryness or flaking of the skin [11–14]. Natural products are preferred by the general population to treat various skin diseases over conventional therapy due to their long history of use and better patient tolerance and safety profiles [15].

The skin microbiota are the resident microbes present in the healthy skin that play an essential role in stabilizing this barrier function by interacting with the immune cells and secreting antimicrobial agents to fight the pathogens [16]. Probiotics, which influence the gut microbiome, also have the capacity to maintain and restore the skin microbiota. However, the application of live bacteria on skin poses several challenges [17]. Postbiotic is a relatively new term used to describe microbial metabolites. These include short-chain fatty acids, extracellular metabolites, functional proteins, cell lysates and other products derived from a probiotic that can influence the microbiome composition [18–20].

Postbiotics have a long shelf life, safety and possess multiple health benefits. They have been evaluated for anti-inflammatory, immunomodulatory, anti-obesogenic, antihypertensive, hypocholesterolemic, antiproliferative and antioxidant benefits [18].

Antimicrobial proteins produced from useful bacteria are one of the expanding fields of research due to conventional antibiotic resistance and are reported to be less toxic than other chemical antimicrobial agents [21–24]. Most of the bacteria, both Gram-negative and Gram-positive, and Archaea produce bacteriocin or bacteriocin-like substances. They are categorized based on their size, structure, mode of action, antimicrobial potency, immunity mechanisms and target cell receptors [25,26]. Bacteriocins are harmless to the human body and the surrounding environment, as they are sensitive to proteases, and have been used as food additives since ancient times [27].

Various research groups have reported the presence of bacteriocins in the *Bacillus* genus [28]. LactoSporin is an extracellular metabolite purified from *Bacillus coagulans* MTCC 5856 fermented broth with an INCI name *Bacillus* ferment filtrate extract. The potential mechanism of LactoSporin as an antimicrobial agent is by pH drop, microbial biofilms inhibition, and draining the ions from the targeted cells [29,30].

In the present study, we report a randomized clinical trial to treat acne in male and female subjects with LactoSporin for 21 days compared with a similar benzoyl peroxide treatment. The antimicrobial activity, thermostability, pH stability and the 5-alpha reductase inhibitory activity were evaluated in vitro to understand the mechanism of action of LactoSporin. The dermatological safety of LactoSporin cream was performed in healthy volunteers by a 24 h primary skin irritation patch test before the efficacy trial.

2. Materials and Methods

2.1. In-Vitro Studies

2.1.1. Bacterial Strains

A total of eight bacterial strains were used in the in-vitro antimicrobial activity of LactoSporin. The reference strains of *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25,922, *Salmonella abony* NCIM 2257, *Streptococcus mutans* MTCC 1943, *Cutibacteriumacnes*(formerly *Propionibacterium acnes*) ATCC 11,827, *Staphylococcus aureus* ATCC 29,213, *Staphylococcus epidermidis* ATCC 14,990 and *Bacillus cereus* ATCC 14,579 were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA), MTCC (IMTECH, Chandigarh, India) and NCIM (National Collection of Industrial Microorganisms, Pune, India). *Bacillus coagulans* SBC37–01 used in the study was characterized and deposited with the Microbial Type Culture Collection, Chandigarh, India, and the strain was assigned as *Bacillus coagulans* MTCC 5856.

2.1.2. Bacterial Medium

S. mutans and *C. acnes* were maintained on a brain heart infusion agar (BHI: Difco Laboratories, Detroit, MI, USA) and reinforced clostridial agar (RCA; Hi Media, Mumbai, India), respectively. *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *S. abony* and *B. cereus* were maintained on trypticase soy agar (Difco Laboratories) at 37 °C. *S. mutans* and *C. acnes* were incubated anaerobically (80% N_2, 10% H_2 and 10% CO_2) at 37 °C for up to 48 h in an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA).

2.1.3. Antimicrobial Activity of LactoSporin (Minimum Inhibitory Concentration)

The antimicrobial activity of LactoSporin was evaluated against *P. aeruginosa*, *E. coli*, *S. aureus*, *S. epidermidis*, *S. mutans*, *C. acnes*, *B. cereus* and *S. abony* using a broth dilution method, as described previously [31,32]. A series of two-fold dilutions of LactoSporin, starting from 8% (*v*/*v*) to 0.015% (*v*/*v*), was prepared in the respective media for each bacterial culture in triplicates. Bacterial suspension in its logarithmic phase (1×10^6 CFU/mL) was used as the inoculum to get a final density of 1×10^5 CFU/mL at 0 h. The viable count was expressed in Log_{10} CFU/mL, and the absorbance was expressed in an optical density value at 610 nm. The viable count and absorbance at 610 nm were taken at 0 h (untreated control) and 24 h of incubation at 37 °C. The minimum inhibitory concentration (MIC_{50}) is the concentration of LactoSporin that resulted in a 50% reduction in OD_{610} compared to the control or a 50% reduction in Log_{10} CFU/mL compared to 0 h.

2.1.4. Effect of pH on Antimicrobial Activity of LactoSporin (pH Stability Study)

The sensitivity of the active substance to different pH was estimated by adjusting the pH of the supernatant samples to pH 2, 3, 4, 5, 6, 7 and 8 with NaOH or HCl and testing against the indicator strain and pathogenic culture by the agar diffusion assay after incubating for 24–48 h. The zone of inhibition was measured in millimeters.

2.1.5. Effect of Temperature on Antimicrobial Activity of LactoSporin (Thermostability Study)

The supernatant was heated at 70, 80 and 90 °C for 30 min to assess heat sensitivity. The heat-treated samples were checked for antimicrobial potency using indicator strain and pathogenic culture by the agar diffusion assay after incubating for 24–48 h. The zone of inhibition was measured in millimeters.

2.1.6. 5-Alpha Reductase Inhibitory Activity of LactoSporin

The 5-alpha reductase inhibitory activity was assessed by the quantity of testosterone converted to dihydrotestosterone in the presence of LactoSporin.

Human follicle dermal papilla cells (HFDPC) were plated at a density of 5000 cells/well (in replicates) in Dulbecco's modified Eagle's medium (DMEM), with 10% fetal bovine serum (FBS) in a 96-well plate. After overnight incubation, the media was changed, and cells were treated with 50-nM testosterone with or without LactoSporin. The cells were incubated for 48 h in a 5% CO_2 incubator at 37 °C. After 48 h, the supernatant was collected from each well. The amount of 5-alpha dihydrotestosterone produced by the cells was quantified in the supernatant using the ELISA method, as per the manufacturer's instructions (DHT ELISA kit from Fine Tests Wuhan, Hubei, China (EU2551-96T)).

Percentage inhibition by LactoSporin in the sample treated wells was calculated compared to testosterone-treated wells by the following formula:

$$\%\ Inhibition\ =\ (C - T)/T \times 100$$

where C = concentration of DHT in the control well (testosterone-treated only), and T = concentration of DHT in the sample-treated wells (testosterone plus LactoSporin).

2.2. Clinical Studies

2.2.1. Test Product

LactoSporin® 2% *w/w* cream was manufactured and provided by Sami Labs Limited Bangalore, India. BENZAC AC gel (2.5% benzoyl peroxide) and neutral cleanser—Cetaphil® cleansing and moisturizing syndet bar were purchased from Galderma Laboratories, LP 14501 North Freeway, Fort Worth, TX 76177, USA.

2.2.2. Primary Skin Irritation Patch Test of LactoSporin

The primary skin irritation patch test was designed to test the dermatological safety of LactoSporin 2% cream on 24 healthy human subjects for 24 h under complete occlusion.

Healthy male and female subjects of 18–65 years having Fitzpatrick skin type III to V and willing to maintain the patch for 24 h and who did not participate in a similar investigation in the past two weeks were enrolled in the study.

Subjects with infection, allergy, antecedents, cutaneous disease, history of excessive sweating, oral corticosteroid or a known allergy or sensitization to adhesives and bandages were excluded from the study. Other exclusion criteria included subjects with a history of underlying medical illness, including diabetes, liver disease or a history of alcoholism, HIV or any other serious medical illness and pregnancy/lactating females.

The test site was between the scapulae and waist of the subject free from pigmentation, pimple, coarse hair, mole or any dermatological conditions that can interfere with the reading. The patch was kept on approximately 24 h and observed for skin irritation parameters at zero hours, 24 h and seven days post-removal.

2.2.3. The Antiacne Potential of LactoSporin

Study Design

This study was a randomized, open-label, comparative, safety and efficacy study. Male and female subjects were screened to enroll 68 subjects, and 30 subjects were expected to complete the study in each arm, considering a 12% dropout rate. The study was conducted for three weeks. During the washout period of 3 to 5 days after the screening, the subjects were instructed to use a neutral cleanser and moisturizing syndet bar twice a day. The subjects were randomly assigned to two groups using computer-generated randomization codes and were dispensed with either LactoSporin 2% or benzoyl peroxide 2.5%, with the direction to use it twice a day for 21 days. A neutral cleanser and moisturizing syndet bar were distributed to both the groups.

Study Population

The study population included male and female subjects of 18 to 35 years, as determined from the recent medical history, general physical examination and dermatological assessment. Subjects with normal-to-oily skin types, Fitzpatrick skin types III to V, with mild to moderate acne on the overall face and not having a hairstyle covering the entire forehead were included in the study. All the subjects included in the study agreed not to use any other product/treatment/home remedy/soap bar on their face, not to carry out bleaching or any other procedures, including facials, or to be exposed to excessive sunlight and to remove all jewelry on/around the face.

Subjects having excessive facial hair, cuts, abrasions, fissures, wounds, lacerations or any other active skin conditions on the face and using systemic medications likely to cause or abate acne were excluded from the study. Subjects with atopic dermatitis, seborrheic dermatitis, skin cancer, actinic keratosis or psoriasis who used topical corticosteroids or hormonal preparations antibiotics or retinoids within the past two weeks on the face were also excluded from the study. Other criteria for exclusion were the use of systemic corticosteroids or antibiotics; facial preparations like abradants, peels, masks, washes, soaps or moisturizers containing glycolic acid, salicylic acid, alpha- or beta-hydroxyl acids or other acids; benzoyl peroxide or sulfacetamide sodium within the past four weeks or during the study period. Subjects not willing to follow the study protocol and having any other condition that may interfere with the study outcome were also excluded from the study.

Study Outcome Assessments

The study's primary outcome measures were the mean reduction in acne lesions as observed by the reduction in elevation and redness by Antera measurement, the mean reduction of sebum secretion through Sebumeter measurement and the mean reduction in noninflammatory and inflammatory lesions by dermatologists' image assessments using VISIA image. The secondary outcome measure was related to the efficacy assessment by a subject self-assessment questionnaire, skin tolerance by dermatologist assessment and product's safety of the product with adverse event occurrence.

The dermatological assessment was performed by counting both the inflammatory and noninflammatory lesions recorded using the assessment form on the baseline (day 0) and days 3, 7, 14 and 21. VISIA CR 2.2 (Canfield Scientific 4, Wood Hollow Road, Parsippany, NJ 07054, USA) was used for facial imaging to record the visual changes. Left, right and front views of the face under cross-polarized and parallel polarized standard 1 (general lighting) and standard 2 (flat lighting) conditions were recorded. Sebumeter® MPA580 (Courage plus Khazaka electronic GmbH Mathias-Brüggen-Str. 9,150,829, Köln, Germany) was used to measure changes in sebum levels on the forehead. Antera 3D™ (MIRAVEX LIMITED, 11 St. Stephen's Green, Dublin, Ireland) was used to measure the acne elevation (medium and small) and redness. Subjects were also given a self-assessment questionnaire to assess the antiacne efficacy of LactoSporin based on oily/greasy skin, pimple, acne spot and redness around acne.

Localized skin irritation was recorded by the dermatological evaluation based on the parameters such as erythema, dryness, edema, urticaria, allergic reactions, etc.

For the patch test of LactoSporin, test sites were assessed for erythema, dryness, wrinkles and oedema, as per the Draize scale for scoring at the treatment.

2.2.4. Ethics and Informed Consent Form

The study was conducted at the MS Clinical Research Pvt. Ltd., Bangalore, Karnataka, India after approval from the Independent Ethics Committee (Clinicom Ethics Committee). It was performed in accordance with the principles stated in the Declaration of Helsinki and its subsequent amendments and the Good Clinical Practice Guidelines. Further, the study was registered prospectively on the clinical trial registry of India with registration number CTRI/2019/10/021562. The primary skin irritation test was conducted as per BIS specification IS 4011:2018 Methods of test for safety evaluation of

cosmetics. The irritation scoring system is, as per clause 4.3.1.3, 4.3.2.6 on the Draize scale for scoring treatment sites. All the subjects of the respective studies signed a freely given informed consent form before enrolment.

2.3. Statistical Analysis

The Shapiro-Wilk test was used for the normality checking of the baseline sample. Parametric values were expressed in mean ± standard deviation/standard error, and nonparametric values were expressed in percentage. Unpaired *t*-test/Wilcoxon signed-rank test for paired samples were used for efficacy assessments within the group and between groups. A two-tailed test was performed for all analyses that used statistical testing. All the statistical tests used a significance level of $\alpha \leq 0.05$.

3. Results

3.1. In-Vitro Antimicrobial, pH Stability, Thermostability and 5-Alpha Reductase Inhibition Studies

The MIC_{50} ranged from 0.5% to 4% for the pathogens tested. LactoSporin was active against *p. aeruginosa*, *S. aureus*, *S. epidermis* and *C. acnes*. LactoSporin is stable and active in highly acidic pH. LactoSporin was found to be temperature-stable up to 90 °C for 30 min. LactoSporin at various concentrations inhibited the 5-alpha reductase enzyme. The percentage inhibition was highest (17.17) at 0.5% *v/v* of LactoSporin (Figure 1).

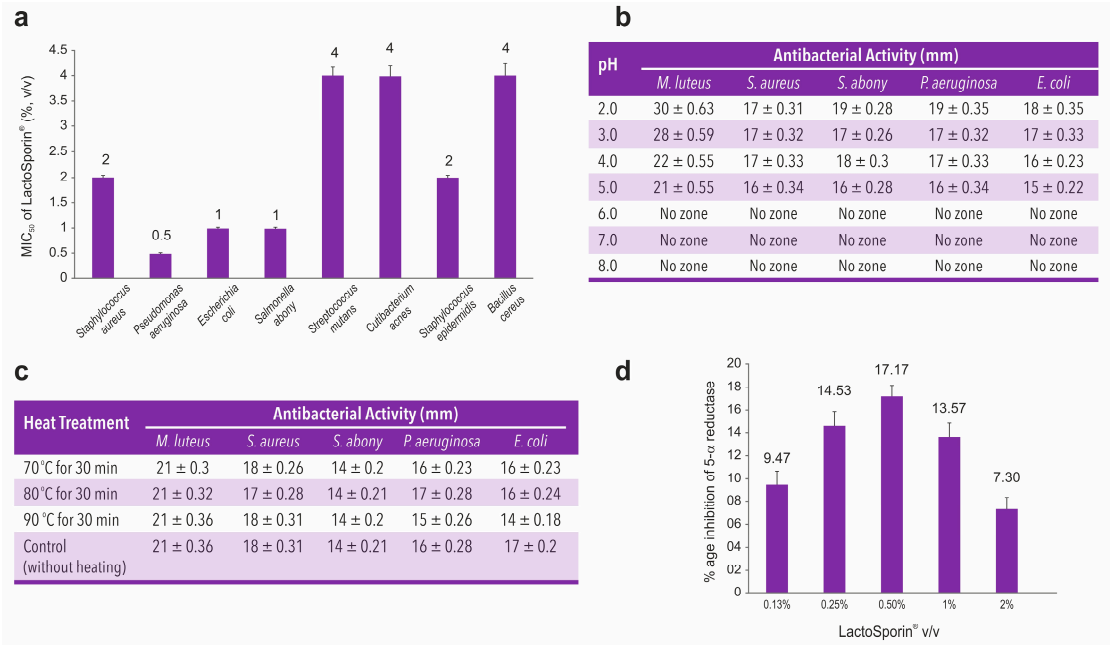

Figure 1. In-vitro studies of LactoSporin. (**a**) Minimum inhibitory concentration (MIC_{50}) of LactoSporin to inhibit the bacterial species, i.e., *Pseudomonas aeruginosa, Escherichia coli*, *Salmonella abony*, *Streptococcus mutans*, *Cutibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis and Bacillus cereus.* (**b**) Table showing the effects of pH on the antibacterial activity of LactoSporin. (**c**) Table showing the effects of temperature on the antibacterial activity of LactoSporin. (**d**) 5-alpha reductase inhibitory activity of LactoSporin. Values are expressed as mean ± SD.

3.2. Primary Skin Irritation Patch Test

LactoSporin 2% cream was deemed a nonirritant and dermatologically safe for the study population compared to the positive control (Table 1).

Table 1. Primary skin irritation test of LactoSporin cream.

SN	Time	LactoSporin Cream		SLS (Sodium Lauryl Sulphate, 1% *w*/*w*)	
		Mean Irritation Score	Irritancy Assessment	Mean Irritation Score	Irritancy Assessment
1	0 h	0.17	Nonirritant	2.13	Irritant
2	24 h	0.04	Nonirritant	1	Irritant
3	Day 7	0.00	Nonirritant	0.79	Nonirritant

3.3. Antiacne Clinical Study

3.3.1. Study Population

In this study, 71 subjects were screened for eligibility, and 68 met the eligibility criteria and were randomized between November 2019 and December 2019. A total number of 68 subjects were enrolled, and a total of 64 subjects completed the trial. Thirty-two subjects completed the study in each arm. The demographics of the samples are shown in Table 2 and Figure 2. Patient demographics and baseline characteristics were similar between the groups. The mean age of the subjects was 24 years, with an equal number of male and female participants.

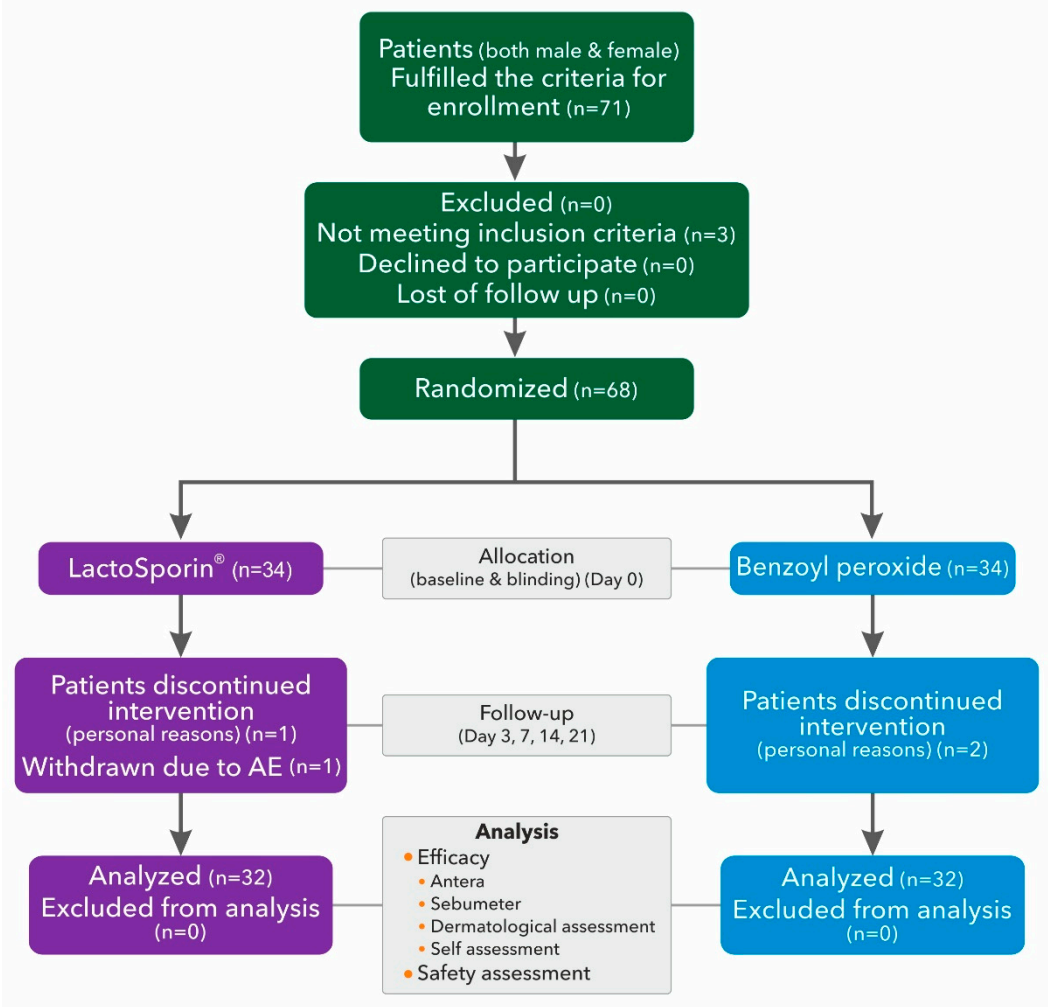

Figure 2. Consort study flow diagram. AE: adverse events.

Table 2. Demographic Details.

Particulars		LactoSporin Cream	Benzoyl Peroxide
Age Mean ± SD Years		23.70 ± 2.07	24.88 ± 2.08
Sex, *n* (%)	Male	18 (53)	15 (44)
	Female	16 (47)	19 (56)
Lesion counts, mean ± SD	Open comedones	1.03 ± 1.82	1.00 ± 1.74
	Closed comedones	33.19 ± 16.76	30.25 ± 15.19
	Papules	7.19 ± 4.72	6.81 ± 4.58
	Pustules	0.53 ± 1.41	0.06 ± 0.25
	Total	41.94 ± 24.71	38.12 ± 21.76
Subjects' skin types, *n*	Normal	12	11
	Oily	22	23

SD—Standard deviation, and *n* = number of subjects.

3.3.2. Assessment Based on the Instrumental Counting of Acne Lesions

Antera 3D™ Assessment

The Antera assessment is a measure of inflammation and was performed on inflamed acne by the dermatologist. Both LactoSporin and benzoyl peroxide showed a reduction in inflammation as early as three days after application. At the beginning of the study, the mean redness in the LactoSporin and benzoyl peroxide groups was 43.89 ± 3.76 and 44.08 ± 2.87, which was subsequently reduced to 39.54 ± 4.55 and 39.18 ± 2.97, respectively (Figure 3a,d,e).

The mean small elevation reduced significantly from 0.36 ± 0.31 mm to 0.14 ± 0.18 mm with a 61.11% decrease in the LactoSporin group and from 0.43 ± 0.32 mm to 0.18 ± 0.16 mm, resulting in a 58.14% reduction for the benzoyl peroxide group ($p < 0.001$ for both) (Figure 3b,d,e). Similarly, the mean medium elevation in the LactoSporin group reduced by 60.93%, and the benzoyl peroxide group reduced by 54%, respectively (Figure 3c–e).

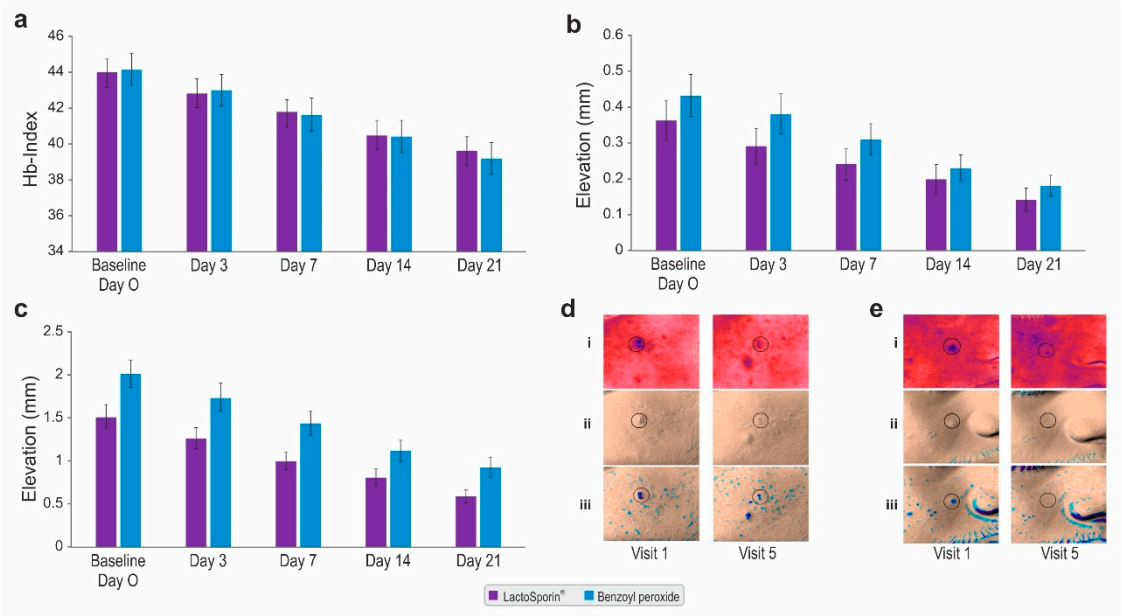

Figure 3. Antera analysis was conducted at day 0, day 3, day 7, day 14 and day 21. (**a**):Redness (Hb-index—% hemoglobin hyper-concentration) in the inflammatory response. (**b**) Elevation (small) in the inflammatory response. (**c**) Elevation (medium) in the inflammatory response. Values are expressed as mean ± SE. (**d**) Antera Images Subject No. 21—benzoyl peroxide: (i) redness, (ii) small elevation and (iii) medium elevation. (**e**) Antera Images Subject No.47—LactoSporin: (i) redness, (ii) small elevation and (iii) medium elevation.

Sebumeter® MPA 580 Assessment—Sebum Secretion

The assessment on the sebum secretion measured via Sebumeter showed a mean value of 106.74 ± 9.78 μg/cm^2 and 108.13 ± 9.32 μg/cm^2 in the LactoSporin and benzoyl peroxide groups, respectively, at the beginning of the study. The same was significantly reduced at the end of the study period (61.41 ± 7.91 μg/cm^2 and 61.11 ± 7.16 μg/cm^2, $p < 0.001$ for both groups) (Figure 4). A significant reduction in sebum secretion was observed at all time points after product application in both the LactoSporin and benzoyl peroxide groups. The percentage of reduction was 42.4% and 43.4% in the LactoSporin and benzoyl peroxide groups, respectively, on day 21 compared to the baseline.

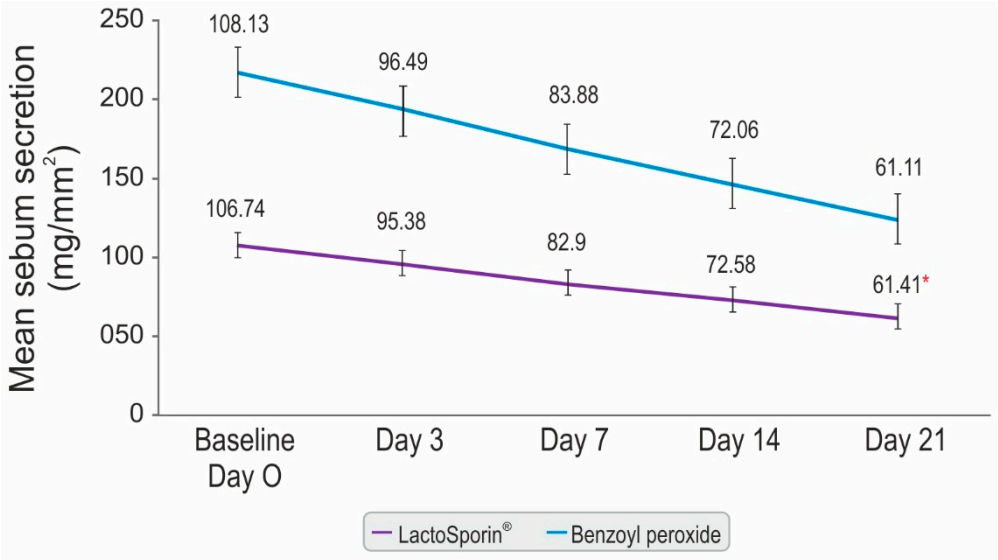

Figure 4. Analysis of sebum secretion (μg/cm^2) by Sebumeter was done at day 0, day 3, day 7, day 14 and day 21. Values are expressed as mean ± SE, * $p < 0.05$ in comparison to benzoyl peroxide.

3.3.3. Dermatological Assessment

The dermatological assessment was performed by counting the inflammatory (papules and pustules) and noninflammatory (open and closed comedones) lesions. The mean number of open comedones reduced from 1.03 ± 1.82 to 0.38 ± 0.75 with a 63.11% reduction for LactoSporin and 1.00 ± 1.74 to 0.50 ± 1.16 with a 50.0% reduction for the benzoyl peroxide group (Figure 5a). Similarly, the closed comedones reduced by 50.02% for LactoSporin and 49.50% for the benzoyl peroxide group (Figure 5b). The significant reduction in open comedones was observed as early as day 7 in the LactoSporin group, while the benzoyl peroxide group was on day 14.

The count of papules reduced following the applications of LactoSporin (69.96%, p <0.001) and benzoyl peroxide (73.86%, p <0.001) for three weeks at all the time points (Figure 5c). The count of pustules reduced from 0.53 ± 1.41 (baseline) to 0.09 ± 0.39 in LactoSporin and from 0.06 ± 0.25 (baseline) to 0.03 ± 0.18 in the benzoyl peroxide group. The resulting percentage reduction was 83.02% in the LactoSporin and 50% in the benzoyl peroxide groups, respectively (Figure 5d).

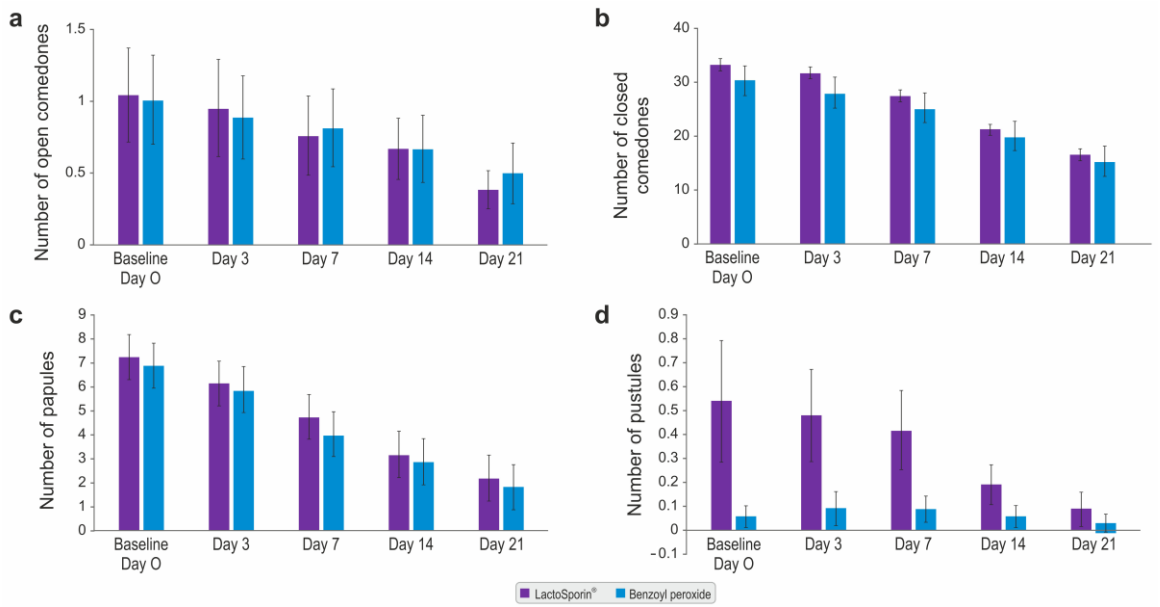

Figure 5. Dermatological Assessment: total number of open comedones, closed comedones, papules and pustules were counted by a dermatologist at day 0, day 3, day 7, day 14 and day 21. (**a**) Count of open comedones. (**b**) Count of closed comedones. (**c**) Count of papules. (**d**) Count of pustules. Values are expressed as mean ± SE.

3.3.4. Assessment Based on Subject Self-Assessment Questionnaire:

The application of both LactoSporin and benzoyl peroxide showed a statistically significant reduction in oil and grease from the face compared with their respective baseline from day 3 onwards, and the perception was more robust on day 21. There was a significant difference noted in the comparison between test products on day 3, day 7, day 14 and day 21, wherein LactoSporin was perceived to be efficacious by a higher percentage of the population than benzoyl peroxide (Figure 6a,b).

Similarly, LactoSporin and benzoyl peroxide were perceived as efficacious in acne, acne spots and redness in 21 days of the treatment (Figure 6c–e).

3.3.5. Safety Outcomes:

One subject, SLAA−034 from the LactoSporin cream group, had increased inflammatory lesions. As per the dermatologist's opinion, the subject was discontinued from the study, and eventually, the respective subject was recovered after taking rescue treatment. There was no serious adverse event (SAE) or local intolerance with the test products, and both test products were deemed overall safe. None of the subjects reported skin irritations, as assessed by the subject self-assessment questionnaire.

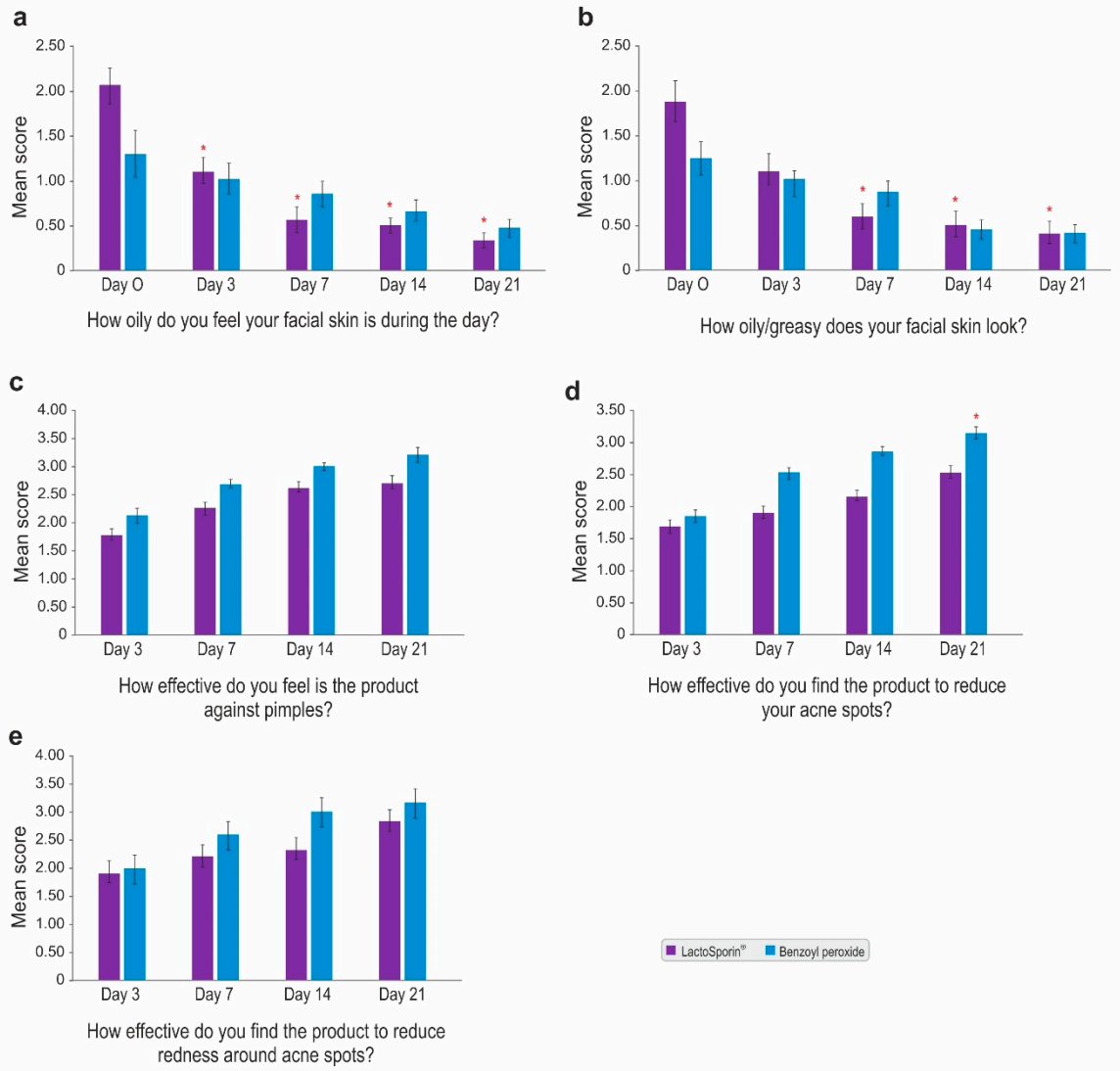

Figure 6. A self-Assessment questionnaire was given to subjects with five different questions to evaluate the improvement in the acne profile at day 0, day 3, day 7, day 14 and day 21. (**a**) How oily do you feel your facial skin is during the day? (**b**) How oily/greasy does your skin look? (**c**) How effective do you feel is the product against pimples? (**d**) How effective do you find the product to reduce your acne spots? (**e**) How effective do you find the product to reduce redness around the acne spots? Values are expressed as mean ± SE, * $p < 0.05$ in comparison to benzoyl peroxide.

4. Discussion

Acne is a multifactorial inflammatory disease, generally known as a disorder of adolescence, which has both physical and emotional impacts on human health [1,33]. The conventional therapies offer only the temporary management of acne and are also associated with undesirable effects. Alternative therapies with minimal observed risks and holistic managements of the problem are in high demand [34,35].

The interaction between skin microbes and host immunity is believed to have a crucial role in acne development. A disturbed skin microbiome, with an over-proliferation of *C. acnes* in sebum-rich areas, contributes to the disease [36]. The clinical manifestation of acne is seen with inflammatory and noninflammatory lesions [37]. Several inflammatory molecules and chemotactic factors are secreted by *C. acnes*, which initiate and perpetuate the local inflammatory response and keratinocyte hyperproliferation [38]. Thus, it is important to treat acne by reducing the inflammation, repairing the skin barrier, maintaining skin hydration and preserving the skin microbiome [37].

The human skin functions as a physical and immunological barrier, providing the first-line defense against external agents. Dysbiosis of the skin microbiome is associated with the aggravation of skin diseases [39]. The comparison of skin microbiota between acne and an acne-free individual has revealed the dominance of *C. acnes* and *S. epidermis*, an increase in Proteobacteria and Firmicutes and a decrease in Actinobacteria [40]. A recent study has demonstrated that treatments with benzoyl peroxide significantly decreased the alpha diversity of skin flora in preadolescent females [41]. Scientific evidence indicates that topical probiotics can modify the skin's pathophysiologic factors that contribute to acne [39].

They also inhibit *C. acnes* by secreting antimicrobial proteins, known as bacteriocins. Studies have shown the efficacy of bacteriocin in inhibiting the inflammatory skin bacteria, such as *S. epidermidis*, *S. aureus*, *S. pyogenes* and *C. acnes* [42]. The bacteriocin activity depends upon its amphiphilic nature, selectivity and ability to disrupt the bacterial cytoplasmic membrane [43,44] while preserving the natural skin homeostasis by altering the skin microflora, skin lipids and immune system [45].

A clinical study of lotion containing bacteriocin produced by *E. faecalis* SL-5 in patients with mild-to-moderate acne lesions caused by *C. acnes* has demonstrated a significant reduction in inflammatory lesions and pustules in comparison to the placebo arm [46]. In the present study, we used an extracellular metabolite (LactoSporin) secreted by *B. coagulans* MTCC5856 as a postbiotic antimicrobial for acne treatment. LactoSporin, manufactured by a unique proprietary process, showed antimicrobial activity against various skin pathogens like *P. aeruginosa*, an opportunistic pathogen, and *S. aureus* and *S epidermidis*, the two most important skin pathogens. Most importantly, it was found effective against *C. acnes*, the acne-causing bacteria, showing a minimum inhibitory concentration of 4%. LactoSporin was thermostable and stable at an acidic pH that is close to the natural pH of the skin. In the clinical scenario, LactoSporin significantly reduced the noninflammatory lesions in both open and closed comedones and improved the signs of inflammation, like redness and skin elevation. The response of the LactoSporin treatment was as early as three days, suggesting its rapid efficacy in relieving the symptoms. Although benzoyl peroxide was also efficacious, the effect of LactoSporin on closed comedones appeared earlier, suggesting an edge over the standard treatment for acne.

At the beginning of puberty, hormonal changes—specifically in the level of circulating androgens—trigger the production of sebum. Seborrhea, due to high levels of sebum, interfere with follicular keratinization, causing pore blockage and, subsequently, lesion formation and acne [47,48]. During this process, the 5-alpha reductase enzyme plays a major role in the overproduction of androgens, testosterone and dihydrotestosterone, which stimulates the secretion of the sebaceous gland favoring the growth of *C. acnes* [49]. Additionally, excessive sebum secretion leaves the skin oily and greasy, and this aids in facial pore development [50,51]. LactoSporin could inhibit the 5-alpha reductase enzyme in vitro, thus reducing the secretion of sebum. In corroboration, the clinical study showed a significantly reduced sebum secretion with the LactoSporin treatment when compared with the standard benzoyl peroxide. LactoSporin was also better than benzoyl peroxide in the improvement of the oily and greasy skin look throughout the study period, implying its 5-alpha reductase enzyme inhibitory activity.

The microbiome is emerging as a major contributor to protect the skin from inflammatory conditions. Postbiotic is a relatively new term to classify the metabolites, cell components derived from a probiotic that can influence the microbiome composition [19,52]. They are bioactive, soluble, nonliving microbial cell products that possess several practical advantages over the probiotic itself, as they show biological activity in the nonviable state and are easier to formulate with favorable physicochemical properties [53]. LactoSporin shows antimicrobial activity at acidic pH conditions. Skin pH ranges from 4 to 6, normally acidic, which suggests that LactoSporin could be highly effective as a topical formulation. Further, it is thermostable and is water-soluble and can be adapted for different formulations with ease. Our results suggest that LactoSporin, the postbiotic from *B. coagulans* MTCC 5856, shows a significant efficacy against pimples, acne spots and erythema by its antimicrobial activity,

as well as by reducing sebum secretion, with a potential role in controlling seborrheic conditions like acne vulgaris.

Study Limitations: This open-label comparative study was conducted at a single site, with a relatively small population.

5. Conclusions

This study suggests that LactoSporin is a postbiotic that is thermostable and stable at an acidic pH. LactoSporin 2% *w/w* cream was a safe antiacne formulation with efficacy comparable to the standard treatment of benzoyl peroxide 2.5% gel. The onset of efficacy was very early for LactoSporin—as early as three days—especially for closed comedones, providing a quicker benefit than benzoyl peroxide. The major finding of this study shows that LactoSporin reduces the sebaceous secretion by its 5-alpha reductase inhibitory and antimicrobial properties, which are better than benzoyl peroxide and can be a potential ingredient for other Seborrheic conditions as well. Considering the clinical efficacy, continuous treatment and patient satisfaction, LactoSporin is highly suitable for treating subjects with mild-to-moderate acne vulgaris. Further studies on a larger sample in multiple ethnic populations may substantiate our findings.

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