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Scalp Sebum Balance: How Jasmine Sambac Extract Adresses Hyperseborrhea to Enhance Scalp Microbiota Harmony

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1. Introduction

Skin homeostasis with an efficient skin barrier is essential to maintain body integrity and hydration which is multifactorial and pending for various elements. Indeed, microbiota composition and equilibrium, stratum corneum structure, fatty acid quantities and composition are key elements for skin health. Fatty acid overproduction, observed in Hyperseborrhea (HS), a prevalent scalp condition, can cause a disruption of this equilibrium. Indeed, HS has emerged as a growing concern due to modern lifestyle factors and environmental stressors. This disorder is socially impactful and excessive scalp sebum production can trigger multiple disorders, including scalp barrier dysfunction and microbiome imbalance [1, 2].

Sebaceous glands are hair follicle–associated exocrine skin glands that continuously release sebum [3]. Sebocytes, the lipid-producing cells within the sebaceous glands, are responsible for synthesizing and secreting sebum, which is essential for skin hydration, barrier integrity, and antimicrobial defenses [4]. Sebum is composed of various lipids, including triglycerides, wax esters, squalene, cholesterol and free fatty acids [5]. The process of sebum production involves the holocrine secretion of sebocytes, where the entire cell disintegrates to release its lipid contents [6]. Excessive sebum production, often associated with conditions such as acne [3, 7] and seborrheic dermatitis, can disrupt skin homeostasis. The overproduction of sebum can also cause pore occlusion, bacterial overgrowth (e.g. *Cutibacterium acnes, Malassezia*), and inflammation, worsening skin and scalp disorders.

Moreover, scalp microbiota interactions and consequently disorders linked to microbiota dysbiosis can be influenced by the capacity of Anti-Microbial Peptides (AMP), part of the host's antimicrobial defenses, to regulate microbiota growth [8] a process crucial for skin and scalp health. To

achieve this regulatory function, keratinocytes synthetize various AMPs such as hβD1, hβD2 and RNAse7 [9], either constitutively or upon induction. In addition, ubiquitous commensal members of the scalp microbiota as *S. epidermidis* and *S. capitis* are known to maintain the composition of a healthy associated skin microbiota by secreting bacteriocins which are also anti-microbial peptides [10, 11].

In this study, we developed a sustainable extract (JSE) from *Jasminum sambac* leaves, a by-product of cultivating this Himalayan shrub commonly known as Arabian jasmine. While the plant's fragrant flowers are prized for religious ceremonies and tea perfuming worldwide, traditional medicine has utilized *Jasminum sambac* for treating conditions like dysmenorrhea, skin diseases, and leprosy, as well as for its analgesic, anti-inflammatory, and sedative properties [12]. We investigated the effect of *Jasminum sambac* extract on scalp sebum control and global regulation of microbiota to ensure scalp health and homeostasis.

2. Materials and Methods

2.1. Jasminum sambac extract (JSE) preparation

A traditional maceration technique was used to develop this active ingredient from *Jasminum sambac* plants and obtain an extract titrated in specific iridoids glycosides (namely sambacosides and molihuasides).

The process begins with *Jasminum sambac* leaves harvested during the summer months from field cultivation, followed by sun-drying. The dried leaves are extracted with a 70% (w/w) ethanol solution. The extraction occurs at room temperature with constant stirring for one hour, using a biomass-to-solvent ratio of 1:10 (w/v). After filtering out the plant material, the ethanol is removed through evaporation. The concentrated extract is then redissolved in 1,3-propanediol solution and filtered to produce a clear final product (JSE) standardized for iridoid glycosides content.

2.2 Evaluation of JSE on lipids production in human sebocytes

Human sebocytes from Cellprogen (#36079-01) at different passages were seeded in a 96-well plate at the concentration of 15.000 cells/well in complemented sebocytes culture medium (SIGMA, #F8205), then allowed to adhere for 24 hours in an incubator at 37 °C, 5% CO_2. After 24h cells supernatants were removed and tested products were added in the presence of oleic acid 10µM for 48h in complemented sebocytes culture medium. Each condition was repeated in four replicates. Salicylic acid is used as a positive reference.

At the end of the incubation period, cells were fixed with paraformaldehyde 10% and stained with BODIPY dye at 5µM (Sigma, #790389) for lipid droplets quantitation and Hoechst at

2µg/ml (Invitrogen, # H3570) for nuclei numeration in PBS during 30min. Images were captured with Nikon Eclipse Ti2 fluorescence microscope on FITC (Bodipy) and DAPI (nuclei) channels with the x20 lens. Quantification of the images was then performed with ImageJ software: Bodipy staining area and nucleus number. Analysis was done by dividing the lipid droplets area by the number of nuclei to obtain an average of total lipid content by cell. The mean and standard deviation for each condition is then calculated. Results were expressed as percentages of oleic acid control condition and were submitted to ordinary one-way ANOVA or Dunnett's multiple comparisons test depending on normality and variance. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

2.3. Evaluation of JSE on anti-microbial peptides expression in human reconstructed epidermis

Reconstructed human epidermis (RHE) were produced with Normal Human Epidermal Keratinocytes isolated from the foreskin of 3 Caucasian donors. The tissues were cultured at the air-liquid interface, for 14 days, in Epilife medium (Fisher Scientific, M-EPI-500-A) containing supplements in order to obtain a fully differentiated epidermis. They were maintained in a humid atmosphere at 37°C with 5% CO_2. After 15 days, RHEs were treated by JSE 0.1% topically. After 72h (D18), 3 replicates of each condition were processed for transcriptomic analysis and 3 other per condition for histological process.

For transcriptomic analysis, total RNAs were extracted using the Qiagen RNeasy kit according manufacturer's recommendation (Qiagen; 74106). The RNA concentration was determined by spectrophotometric measurement (QIAxpert, Qiagen) and the RNA quality was analyzed by capillary electrophoresis (Agilent Bioanalyzer 2100 - Agilent RNA 6000 Nano Kit, 5067-1511). Reverse transcription of total RNA to complementary DNA (high-capacity RNA-to-cDNA kit, Applied Biosystems; 438706) and qPCR (TaqMan Fast Advanced Master Mix, Applied Biosystems, 4444557) were performed according to the manufacturer's instructions. Threshold cycles (Ct) were obtained for each gene. Results were analyzed using the *DataAssist* Software (v3.0, *Applied Biosystems*) designed to perform relative quantification of gene expression using the comparative Ct ($\Delta\Delta$Ct) method through a combination of statistical analysis.

For histological analysis, RHEs were rinsed with PBS, fixed in 4% formaldehyde solution, dehydrated and embedded in paraffin. Sections of 5µm thick were generated using a microtome (MM France, HM340E) and laid over microscopic slides. One section of each sample was dewaxed, rehydrated and permeabilized (Triton x100 0.5% in PBS, 30min, 37°C). AMPs were immunostained using the following primary antibodies (all at 1/100 dilution):

- anti-RNAse7 antibody (abcam ab205565,) or anti-Psoriasine antibody (abcam ab13680), both revealed with Alexa fluor® 488 -conjugated antibody (Invitrogen A11029, 1/1000)

- anti-hβDF1 antibody (abcam ab203307) or anti- hβDF2 antibody (abcam ab63982) both revealed with Alexa fluor® 568-conjugated antibody (Invitrogen A11011, 1/1000).

Immunofluorescence pictures were taken with a Nikon microscope Eclipse Ti2 and quantified by ImageJ software. Analysis was done by dividing the obtained specific fluorescent intensity by the number of nuclei counted in the epidermis to obtain an average of normalized intensity per cell count. The mean and standard deviation for each condition is then calculated. Results were expressed as percentages of RHE control condition and were submitted to paired T test comparisons. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

2.4. Evaluation of JSE on bacteria growth

100 µL of liquid medium is placed in each well of a sterile 96-well microplate, except for the first column. 200 µL of 4% solution is placed in the first well of two rows of the microplate, and 2-fold dilutions are made in columns 1 to 10 inclusive. The entire microplate is seeded by subculturing 1 to 3 µL of bacteria suspension (*S. epidermidis, S. aureus, S. hominis, P. pararuginosa, C. acnes*) in each well, except for column 11 (sterility control). The microplates are incubated in an appropriate culture medium. The MIC of a product is defined visually as the lowest concentration without growth.

2.5. Evaluation of JSE effects on human volunteer's scalp

2 clinical studies were conducted on volunteers aged from 18 to 70 years old presenting an oily scalp.

- The first study aimed to evaluate the effect of the extract on serum formula, on 34 volunteers (women) with sensitive scalp, applying the serum containing JSE at 0.25% (15 vol) or the placebo (19 vol) (Water, Sodium Polyacryloyldimethyl Taurate, Citric Acid, Sodium Citrate, Phenoxyethanol, Ethylhexylglycerin, Polysorbate 20, Alcohol Denat., Fragrance) once daily in the evening for 28 days.
- The second study focused on a rinsed off shampoo formula, applied three times per week on 36 volunteers with irritated scalp, for 28 days, containing JSE at 0.25% (18 vol) or placebo (18 vol) (Water, Citric Acid, Sodium Citrate, Sodium Benzoate, EDTA, Cocamidopropyl Betaine, Glycol Stearate, Laureth-4, Cocoyl Methyl Glucamide, Sodium Laureth Sulfate, Guar Hydroxypropyltrimonium Chloride, and Citric Acid).

For both studies, sebum level was assessed at day 0 and day 28 by Sebumeter® (Courage + Khazaka electronic GmbH). For the shampoo study, scalp microbiome was analysed from swab sampling using next-generation sequencing (NGS) via an optimized protocol (GenoScreen GenoBiome® Skin).

3. Results

3.1. JSE reduces lipids over production by human sebocytes

In our *in vitro* model of sebocyte lipids production, oleic acid (OA), a monounsaturated fatty acid present in sebum, is used as a stimulant. OA promotes the formation and accumulation of lipid droplets within sebocytes, mimicking the process of sebum production observed *in vivo* [13]. Figure 1 showed, as expected, that OA induced a statistically significant lipid overproduction (+93%***) compared to untreated condition. Salicylic acid [12] significantly decreased (-26%***) lipid content under OA induction.

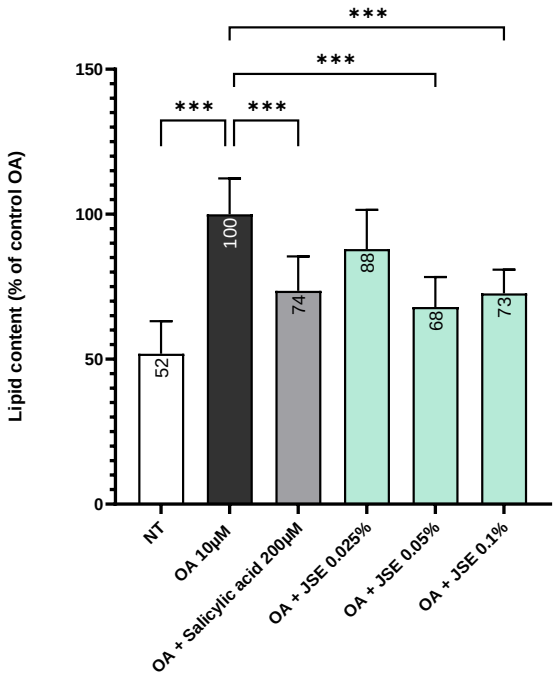

Figure 1: **Sebocyte lipid production (% of the OA condition after JSE addition or not).** OA at 10µM was used as positive control of induction. Salicylic acid at 200µM was used as a reference to abolish OA induction. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

JSE significantly inhibited lipid production under OA induction of 27%*** at 0.1% and 32%*** at 0.05% showing the same efficacy as the salicylic acid benchmark. No significant activity was observed at 0.025%.

3.2. JSE promotes AMP expression

At gene level, JSE at 0.1% was able to increase significantly hβD1 and RNAse7 genes expression by 40%* and 170%** respectively (Figure 2).

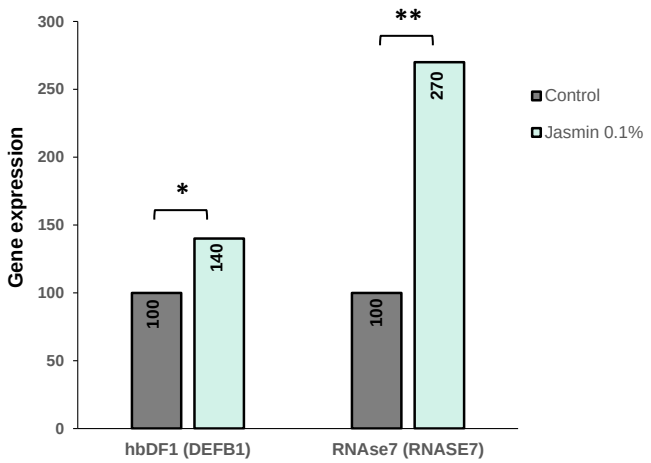

Figure 2: hβD1 and RNAse7 genes expression versus control (normalized percentages – 72h after RHE treatment with or without JSE 0.1%). Ct values were normalized to the Ct of 2 housekeeping genes (YWHAZ and RPLP0) and analyzed with DataAssist Software. The statistical significance value is p<0.05 (*p<0.05, **p<0.01, ***p<0.001).

At a protein level, JSE was able to enhance basal AMP expression in reconstructed human epidermis (hβD1, hβD2, Psoriasin, RNAse7) by +44%***, +82%***, +31%* and +305%*** respectively (Figure 3).

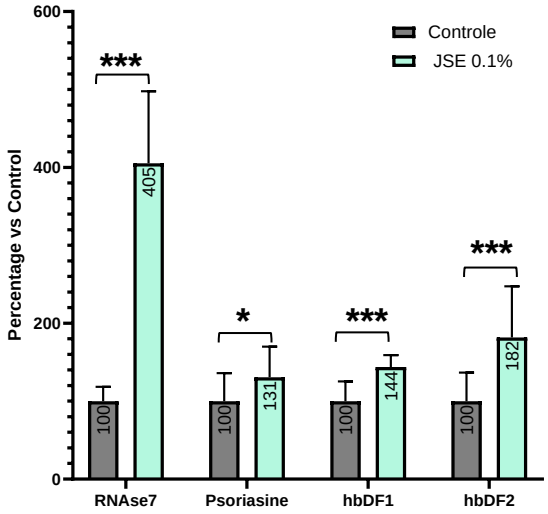

Figure 3: AMP proteins expression versus control (normalized percentages – 72h after RHE treatment with or without JSE 0.1%). Immunofluorescence staining pictures were quantified by Image J software. The statistical significance value is p<0.05 (*p<0.05, **p<0.01, ***p<0.001).

3.3. JSE does not impact bacteria growth

No impact of JSE was noticed on growth inhibition on common human skin bacteria i.e. no Minimal Inhibitory Concentration (MIC) lower than 4% the highest evaluated concentration (Table 1).

Organisms / MIC	*Staphylococcus epidermidis* DSMZ 28764	*Staphylococcus aureus* DSMZ 799	*Staphylococcus hominis* DSMZ 20328	*Pseudomonas paraeruginosa* DSMZ 1128	*Cutibacterium acnes* CIP 53.117T
Propanediol 80%	>4%	>4%	>4%	>4%	>4%
JSE	>4%	>4%	>4%	>4%	>4%

Table 1: MIC determination. 5 commensal bacteria species (*S. epidermidis, S. aureus, S. hominis, P. pararuginosa, C. acnes*) of human skin were evaluated.

3.4. JSE reduces scalp hyperseborrhea and balances scalp microbiome *in vivo*

When applied as a serum at 0.25% of JSE (Figure 4), the sebum production decreased by 45% at Day 28 ($p < 0.05$). In shampoo form, the same concentration of JSE also significantly reduced excess of sebum after 28 days, with a 45% decrease observed ($p < 0.01$ vs. baseline; $p < 0.1$ vs. placebo), whereas no significant change was observed in the placebo groups in both studies. These consistent results across two different application forms—serum and shampoo—highlight the versatility of JSE and its strong potential to restore scalp balance in a variety of cosmetic formulations.

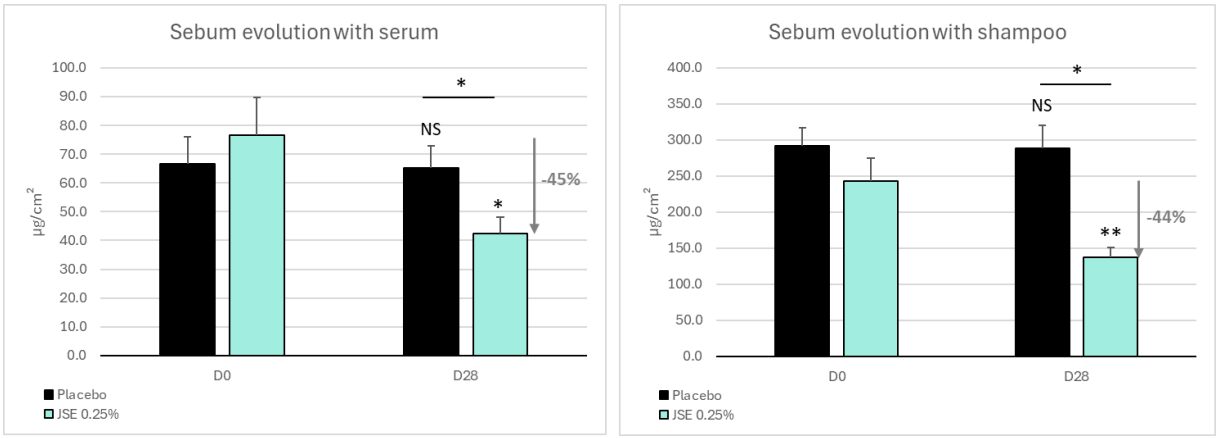

Figure 4: Sebumetric results of JSE in a serum and in a shampoo after 28 days of use. Statistics: ** :p<0.01; *: p<0.05; NS: non-significant.

With the shampoo formula, after 28 days of use, JSE significantly increased the alpha diversity of the scalp microbiota compared to the baseline ($p < 0.005$) (Figure 5A), indicating a richer and more balanced microbial ecosystem. This effect was not observed in the placebo group (# vs. placebo). In addition to this improvement in diversity, JSE reduced inter-individual variability and microbial heterogeneity across volunteers. At the species level, a significant increase in *Staphylococcus capitis* relative abundance was observed in the JSE group at Day 28 compared to Day 0, an effect not seen with the placebo (Figure 5B).

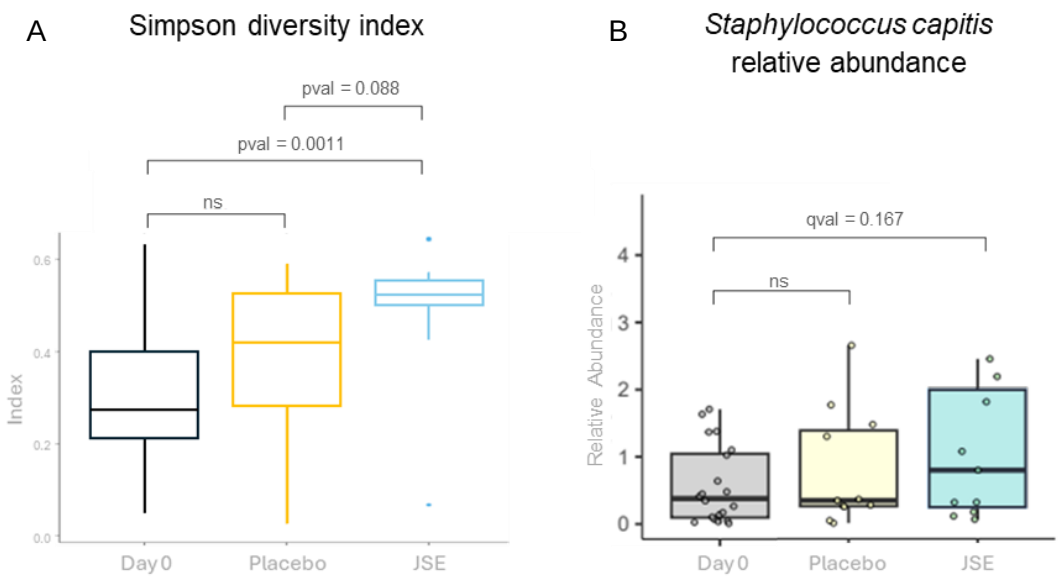

Figure 5: Effects of a JSE shampoo formula on scalp microbiota after 28 days of use. (A) Simpson alpha diversity index. Significance was defined by p-value < 0.1.(B) Relative abundance of Staphylococcus capitis. Significant associated species with groups were calculated with GLM analysis (Taking account cofounding factors: extraction batch number, storage delay, age, gender, hair type). Significance was defined by q-value < 0.25.

These results highlight the ability of JSE to restore scalp microbiota balance, promoting a healthier microbial environment.

4. Discussion

JSE emerges as a potent, sustainable natural active ingredient able to maintained a healthy scalp through multiple mechanisms. First, it reduces sebocytes fatty acid production as evidenced by both in *in vitro* and *in vivo* studies. Second, while JSE does not directly alter human microbiota populations (as demonstrated by the of absence of growth inhibition against common scalp bacteria), it appears to preventively maintain scalp homeostasis by enhancing endogenous defense mechanism. Specifically, JSE stimulates increased expression of antimicrobial peptides (AMPs) [14, 15] thereby preventively preserving scalp microbiota equilibrium through AMPs-mediated regulation of microbial growth processes [8].

Sebum affinity is highly variable among skin microbiota strains, indicating that sebum metabolism is a key factor shaping the scalp microbial community [16]. By reducing scalp HS,

JSE promotes a more balanced microbiota, as shown by an increased diversity index after 28 days of treatment. Additionally, JSE supports the growth of *Staphylococcus capitis*, a commensal coagulase-negative staphylococcus (CoNS) known to act as “host guardians” by targeting pathogens [11]. Together with other commensals like *S. epidermidis, S. capitis* contributes to scalp health through the production of bacteriocins that help control opportunistic pathogens such as *Malassezia* [17]. Thus, JSE exerts a dual effect – regulating sebum and reshaping the microbiota – by increasing diversity and promoting beneficial bacteria, ultimately harmonizing the scalp ecosystem toward a healthier profile.

To further elucidate JSE's impact on HS, advanced models mimicking disorder-promoting factors will be developed, including environmental (pollution), hormonal (testosterone, progesterone), and stress-related (cortisol) variables. Additional investigations will examine JSE's effects on *S. capitis*, specifically focusing on strain growth dynamics and secretome composition (particularly bacteriocin production). Furthermore, the potential benefits regarding sebum regulation and hair greasing will be assessed through a prospective clinical trial.

5. Conclusion

In conclusion, our studies highlight the dual efficacy of JSE in regulating sebum overproduction and enhancing the skin-scalp's natural anti-microbial defenses without the ability to kill directly the major skin commensal bacterias. This innovative approach not only reduces scalp oiliness but leads to overall skin-scalp health promotion by rebalancing/controling the microbiota. JSE emerges as a potent, sustainable natural active ingredient that offers a transformative solution for oily skin-scalp concerns, setting a new standard in hair and scalp care.

6. References

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