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“Characterization of the axillary skin as a support for the development of cosmetic formulations for the control of hyperpigmentation”

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1. Introduction

The axillary skin is continuously exposed to various physical, chemical, and biological factors that can compromise its integrity, often triggering inflammatory responses and leading to post-inflammatory hyperpigmentation (PIH) [1, 2]. Hair removal methods such as shaving, waxing, and laser treatment, along with the frequent use of deodorants and antiperspirants, and the friction caused by skin-to-fabric contact, are among the main causes of irritation in this area. Chronic inflammation resulting from these stimuli can stimulate melanocyte activity, increasing melanin production and consequently exacerbating PIH, ultimately affecting the evenness of the axillary skin tone [2, 3].

Beyond the aesthetic discomfort and its psychological impact on self-esteem, PIH is associated with persistent inflammatory responses, highlighting the need for effective strategies to manage this condition [4]. The development of cosmetic formulations targeting PIH must go beyond pigment modulation and address the maintenance of skin barrier homeostasis and the hydrolipidic balance of axillary skin. However, to ensure efficacy, it is essential to understand the structural and morphological and physiological characteristics of this specific region, which directly influence the skin response to cosmetic treatments and underscore the importance of a tailored approach [5,6].

Active ingredients from natural sources, particularly those with antioxidant and anti-inflammatory properties, offer promising alternatives for modulating the mechanisms involved in skin pigmentation [5]. In this context, a standardized blend of Mediterranean plant extracts - Citrus Sinensis (Orange) Fruit Water, Oryza Sativa (Rice) Extract, Capparis Spinosa Fruit Extract, Olea Europaea (Olive) Leaf Extract, and Maltodextrin, has shown potential in addressing PIH. Rich in polyphenols with well-documented antioxidant and anti-inflammatory activity, this blend may help regulate pigmentation by neutralizing oxidative stress and inhibiting tyrosinase, a key enzyme in melanin biosynthesis [7]. In this context, the objective of the present study was to characterize hyperpigmented axillary skin and developed cosmetic formulations added with a blend of Mediterranean extracts to reduce hyperpigmentation and keep the skin hydrolipidic balance in the skin axillary region.

2. Materials and Methods

2.1. Skin Characterization

This study was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo (CAAE: 83685624.4.0000.5403) and was carried out according to the Declaration of Helsinki.

Twenty healthy female participants, aged between 18 and 30 years, Fitzpatrick skin phototypes II to IV and presenting post-inflammatory hyperpigmentation (PIH) in the axillary region, were recruited. All participants underwent a 20-minute acclimatization period in a controlled environment ($22 \pm 3^\circ\text{C}$ and $45 \pm 5\%$ relative humidity) prior to measurements.

The following parameters were evaluated in the lesional (with PIH) and non-lesional (without PIH) axillary region: stratum corneum water content, sebum amount, transepidermal water loss (TEWL), skin microrelief, porphyrin quantification, melanin and erythema index, and skin morphologic characteristics.

Instrumental Measurements

Sebum Content

Sebum levels were measured using the Sebumeter® (Courage & Khazaka Electronic, Germany), which quantifies sebaceous secretion based on photometric analysis of grease spot intensity, independent of moisture interference [8].

Porphyrin Quantification

Porphyrins were visualized using the Visiopor® PP 34 camera (Courage & Khazaka Electronic, Germany), which uses specific ultraviolet light to detect fluorescence associated with Propionibacterium acnes in lesions, including comedones, papules, and pustules. The analysis covers a minimum area of $8 \times 6.4 \text{ mm}$ [8].

Transepidermal Water Loss (TEWL)

TEWL was measured using the Tewameter® TM (Courage & Khazaka Electronic GmbH, Germany), based on the Fick diffusion principle. The results are expressed in $\text{g/m}^2/\text{h}$ [8].

Melanin and Erythema Index

Skin pigmentation and erythema were quantified using the Mexameter® MX16 (Courage & Khazaka, Germany), a colorimetric device recognized for its precision in skin chromophore analysis [9].

Skin Morphological Characteristics

The characteristics of the different layers of the epidermis were evaluated by Reflectance Confocal Microscopy (RCM) - Vivascope® 1500. From the images obtained, the following parameters were analyzed: epidermis morphology organization of the keratinocytes, brightness of the basal and granular layers, and pigmentation pattern. A qualitative score (0–3) was applied to assess epidermal morphology, based on four criteria: stratum corneum reflectance, furrow size, inter-keratinocyte brightness, and basal layer brightness related to pigmentation. [5,9,10].

Data Analysis

All experimental data were statistically analyzed using GraphPad Prism 8.4.3 (GraphPad Software, San Diego, USA). Results were considered statistically significant at $p < 0.05$.

2.2. Formulations

A cosmetic O/W emulsion based on Cetearyl Alcohol (and) Ceteareth-20, Caprylic/Capric Triglyceride, glycerin and Xylityl Sesquicaprylate was developed without (vehicle - F1) or with the Mediterranean extract blend (F2).

2.3. Texture Profile and Spreadability

Texture and spreadability parameters were evaluated using a Texture Analyzer TA.XT Plus® (Extralab Brasil, Brazil) with Exponent software. Texture was assessed using the Back Extrusion Rig (A/BE) probe (35 mm diameter). Parameters measured included firmness, consistency, cohesiveness, and viscosity index [11]. Spreadability was evaluated using the TCC Spreadability Rig (HDP/SR). The work of shear was determined by calculating the area under the positive force-time curve, reflecting the force required to spread the product [11, 12].

2.4. Sensory Evaluation and Perceived Efficacy

Sensory analysis was performed with 30 healthy participants, aged 18–30 years. Participants applied 80 µL of the assigned formulation to the axillary region, performing 20 circular movements. Immediately after application, participants completed a questionnaire assessing spreadability, texture, and stickiness.

To assess perceived efficacy, 20 healthy women, 18–30 years old) with PIH in the axillary region were recruited and randomly assigned to two groups (F1 or F2). Both formulations were applied once daily at night following hygiene routines. After 7 days of use, participants completed a self-assessment questionnaire to report their perception of the formulation efficacy.

3. Results

The results of skin characterization analysis showed that the lesional area (with PIH) presented significantly higher sebum content compared to the non-lesional area (without PIH) (Figure 1). Additionally, the size of porphyrins was also significantly greater in the lesional region (Figure 2).

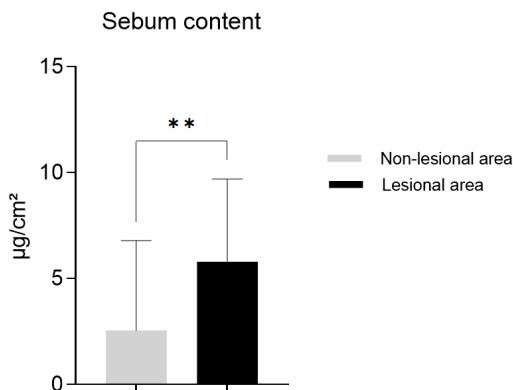


Figure 1. Sebum content in non-lesional region and in lesional region

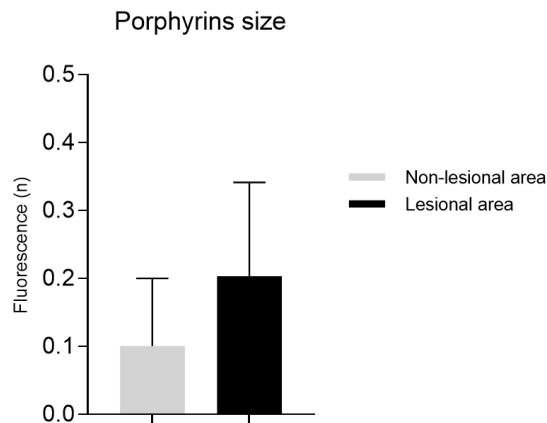


Figure 2. Porphyrin size in non-lesional region and in lesional region

No statistical differences were observed between the regions in terms of transepidermal water loss (TEWL). However, significantly higher values of erythema index and melanin index were found in the lesional area (Figure 3), indicating increased inflammatory and pigmentation activity.

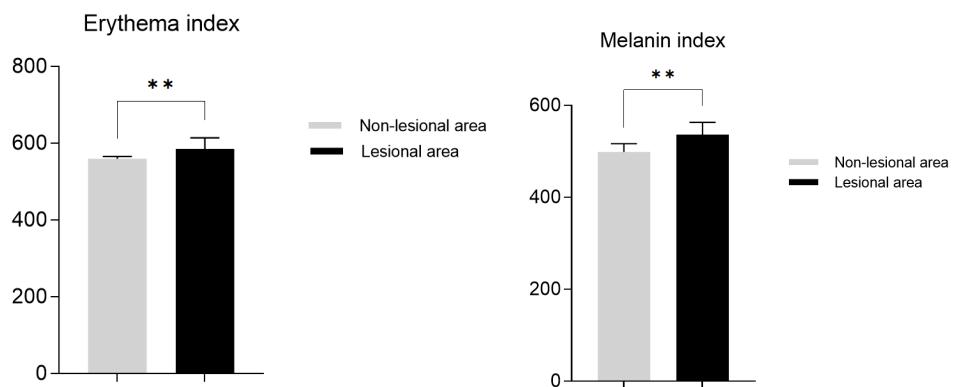


Figure 3. Erythema and melanin index in non-lesional and lesional areas.

The Reflectance Confocal Microscopy imaging analysis showed higher reflectance in the basal layer, indicating the melanin deposition in the lesional region. The non-lesional region showed reduced furrow size, better furrow morphology and higher stratum corneum reflectance, which suggests skin hydration.

Regarding the development of cosmetic formulations, the spreadability test showed a significantly lower work of shear in formulation F2, when compared to F1, (Figure 4). Similarly, the texture analysis showed a significantly lower viscosity index for formulation F2 (Figure 5).

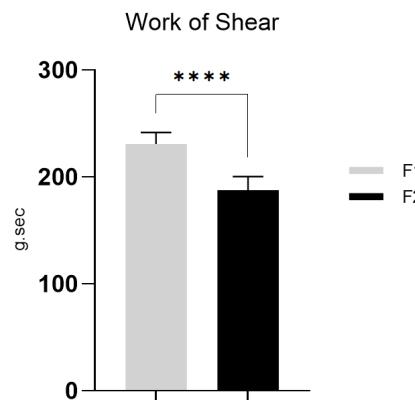


Figure 4. Work of shear of the formulations with (F1) or without (F2) the Mediterranean extract blend, 24 hours after preparation at room temperature.

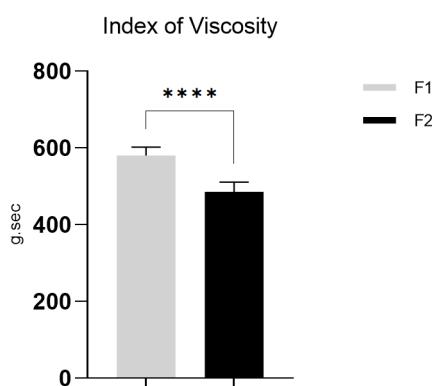


Figure 5. Index of viscosity of the formulations with (VA) or without (V) the Mediterranean extract blend, 24 hours after preparation at room temperature.

In the sensory analysis the formulation was well tolerated and showed good acceptability among the participants. Most participants rated spreadability as "good" to "excellent," with over 90% agreeing or totally agreeing that the product had good spreadability. Regarding texture, approximately 80% of users agreed or totally agreed that the formulation presented a pleasant texture. The sticky sensation was reported as low or absent since the majority of participants disagreed or totally disagreed with the statement that the formulation left a sticky feeling, indicating a positive sensorial profile (Figure 6).

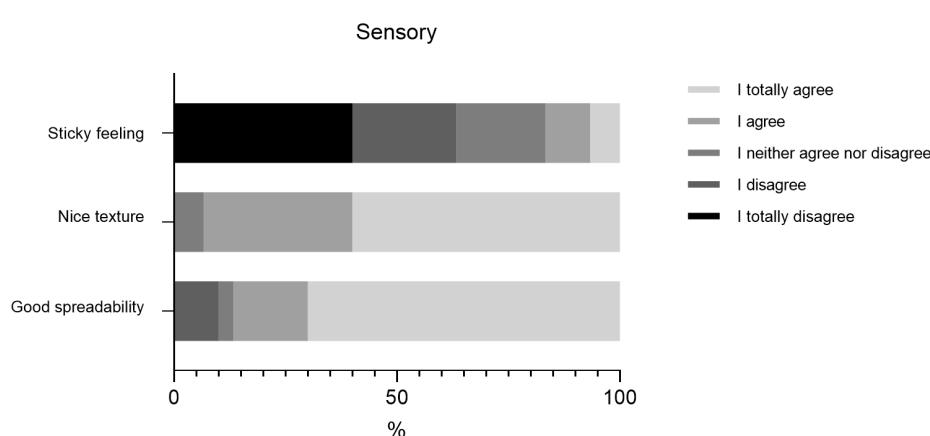


Figure 6. Sensory analysis of the studied formulation

Regarding the perceived effectiveness after seven days of use, participants in both groups reported improvements in the overall appearance of their skin and hydration. However, the group that used formulation F2 — containing the blend of Mediterranean plant extracts — showed a higher percentage of positive responses in terms of reduction in the area and intensity of hyperpigmentation in the armpits.

4. Discussion

The significantly higher sebum content observed in the lesional area corroborated with previous studies indicating that areas affected by PIH often present increased sebaceous activity, which may contribute to follicle obstruction and subsequent inflammation [3,10]. Similarly, the increased porphyrin content in the lesional region suggests an imbalance in the cutaneous microbiota, probably due to increased colonization by *Cutibacterium acnes*, a condition associated with post-inflammatory events [3].

Although TEWL did not differ significantly between regions, the elevated erythema and melanin indices in the lesional areas confirm the presence of inflammatory and hyperpigmentary activity. These findings are in line with other studies demonstrating that PIH is often characterized by epidermal and dermal pigment deposition secondary to inflammatory mediators and oxidative stress [1,2,5].

The evaluation of the formulations showed that the presence of the Mediterranean extract blend in F2 significantly reduced shear stress and viscosity index, indicating better spreadability and a lighter sensory texture. These rheological properties are directly correlated with greater consumer acceptance and a better application experience [11,12].

In the sensory analysis, the formulation was evaluated as having good spreadability and a pleasant texture, being less associated with a sticky sensation. These attributes are essential for cosmetic formulations intended for the axillary region, particularly in the treatment of post-inflammatory hyperpigmentation (PIH).

The skin characterization data revealed that hyperpigmented axillary areas tend to have higher sebum content, which can lead to the perception of oiliness and discomfort when occlusive or heavy formulations are applied. Therefore, the light, non-greasy and non-sticky sensory profile reported by users becomes a highly desirable characteristic in this context. A well-absorbed, easy-to-spread, residue-free formulation not only increases user comfort and compliance, but can also contribute to skin homeostasis, reducing the risk of friction and inflammation that can aggravate hyperpigmentation.

After seven days of application, the group using formulation F2 reported improvements in the reduction of visible skin hyperpigmentation due to the Mediterranean extract blend added to this formulation, which is rich in polyphenols and present known antioxidant and anti-inflammatory effects. Specifically, polyphenols such as those found in *Citrus sinensis* and *Olea europaea* have been shown to inhibit tyrosinase activity, thereby reducing melanin synthesis and contributing to the attenuation of hyperpigmented lesions [7]. Furthermore, the antimicrobial and anti-inflammatory properties of these compounds may aid in reducing persistent erythema and help rebalance the microbiota, contributing to the overall improvement of skin tone and restoration of skin barrier integrity [6,12].

5. Conclusion

The hyperpigmented skin area has a higher amount of melanin and showed alteration in the skin hydrolipid balance compared to the non-lesional area. The proposed formulation showed suitable sensory properties for application in the axillary region, and the blend of extracts improved its spreadability.

The formulation containing a standardized blend of Mediterranean plant extracts (F2) showed better rheological, texture and sensory properties when compared to vehicle formulation (F1). Sensory analysis confirmed good acceptability, and study participants

perceived a reduction in skin hyperpigmentation after seven day-period of F2 daily application.

These findings contribute to a better understanding of the characteristics of hyperpigmented axillary skin and support the development of more specific and effective formulations containing an innovative blend of natural extracts from the mediterranean for treatment of axillary skin with PIH.

6. References

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