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EFFECTS OF NATURAL-ORIGIN COSMETICS ON SKIN PORES AND ANTIOXIDANT CAPACITY

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INTRODUCTION

Enlarged facial pores are surface-level skin structures that manifest as small openings on the skin corresponding to the openings of the pilosebaceous apparatus. These openings can become blocked with horny follicular plugs made up of sebaceous debris, which appear as open comedones. The term "skin pores" is a colloquial expression that lacks precise medical definition and is often categorized based on arbitrary circular diameters determined using cosmetic skin analyzers. In medical terms, it pertains to the enlargement of pilosebaceous follicles, with or without the presence of open comedonal horny impactions, which can be observed with the naked eye. These enlarged pores are most frequently found on the face and scalp and remain a common cosmetic concern that can affect a patient's quality of life. Treatingenlarged pores is challenging, in part due to our limited understanding of the underlying causes. Therefore, in this review, we will explore the currently proposed factors contributing to the enlargement of pilosebaceous openings and discuss treatments within the context of this pathogenesis, with a specific focus on their therapeutic effectiveness. In this study, we assessedthe pore-improving effects of Monteranc Baby Pore Essence by observing 24 female patients with enlarged pores over a specified period.

MATERIALS AND METHODS

We conducted a study on 24 female patients with enlarged pores who presented themselves for treatment. The patients' faces were divided into two halves, with one half receiving the application of the Monteranc Baby Pore Essence, while the other half did not. The application

was done twice daily for two weeks, and measurements were taken before application, the day after application, one week later, and two weeks later. Measurements were taken in the butterfly zone, T-zone, and cheek area. Specifically, we measured pore count, pore volume, blackheads(whiteheads), keratin assessment, sebum and oil content, skin texture (roughness), skin moisture, and skin brightness using widely recognized methods.

Skin photography

To assess changes in pore size, wrinkles, and brightness, we used the RSA (Robo Skin AnalyzerCS50, Inforward Inc., Japan).

Pore Count and Volume assessment

To evaluate changes in pore count and volume, we employed PRIMOS Lite (field of view 18x13 - simple, flexible 3D measuring, GFMesstechnik GmbH, Germany) to capture three consecutive images of the left nasal area of the test subjects. To prevent movement during measurements and to ensure that pore contraction or relaxation did not occur at the measurement site, the test subject's face was securely held in a specially designed PRIMOS facial fixation device set. Furthermore, for measurement reproducibility, we adjusted the measurement area of the test subject's left nasal area to align with the focus pattern of PRIMOS Lite before conducting measurements. Measurements with the device were conducted at two time points for both the control and experimental groups: before treatment and after treatment. The captured images were analyzed using PRIMOS Lite software (PRIMOS Lite version 5.6E, Germany). After applying 3D matching, the matched measurement area was used for analysis. Pore analysis was performed by calculating variable values using volumes of rising. These variable values were measured in μm^3 and indicated an increase in volume as the value increased.

Evaluation of blackheads

To assess changes in blackhead pore conditions, we utilized the full-face imaging system, RoboSkin Analyzer CS50 (Inforward, Inc., Japan). After capturing the entire face, we zoomed in onspecific areas for analysis. To ensure consistent imaging, we fixed the test subject's face in a frontal position beneath a cage with uniform lighting and the same positioning. Measurements with the device were conducted at two time points for both the control and experimental groups: before treatment and after treatment. For precise reproducibility, we overlaid key points from pre-treatment facial images with post-treatment images to compare the results. Additionally, we visually assessed blackhead pore conditions by enlarging and

editing photos of the treated area, the nasal area.

Assessment of keratin levels

To evaluate changes in skin keratin levels, we employed Keratin stickers and a Digital light microscope (Advanced Microscopy Group Inc., USA). Before treatment, we collected keratin samples from the right nasal area of both control and experimental group subjects using Keratin stickers. After treatment, we collected keratin samples from the left nasal area and stained them with Coomassie brilliant blue G (Sigma-Aldrich, Inc., USA) for 24 hours. Subsequently, we removed excess dye and rinsed the samples to ensure that only keratinized cells were stained. Stained keratinized cells were then captured at 100x magnification using the camera built into the microscope. We visually assessed keratin improvement using the "Standard Keratin Score,"ranging from score 1 to 5 (score:
1=very mild, 2=mild, 3=moderate, 4=severe, 5=very severe).

Rolling method

The evaluation of blackheads (sebum) and keratin levels was carried out using the rolling method. In this method, without cleansing the face, a sufficient amount of the Baby Pore Essence was applied to the fingertips and gently massaged into the skin for 5 minutes. Following this, a small amount of water was applied to the hands, and rolling was performed in the opposite direction. The amount of sebum removed during this process was measured, and the remaining keratin on the face was assessed after the rolling method.

Evaluation of Oil and Sebum

To assess changes in skin oil content, we utilized the Dermalab USB sebum probe (Cortex Technology, Inc., Denmark), and the analysis was performed using the dedicated Application software version 1.09. The measurement method involved pressing the sebum collection strip onto the right nasal area of the test subjects with consistent pressure for 30 seconds to absorb the oil. Then, the sebum collection strip was inserted into a Tapereader device to calculate the degree of transparency caused by the oil, which allowed for measurement. This provided a percentage indication of the oil's saturation level. For quantifying sebum, we used a Digital light microscope (Advanced Microscopy Group Inc., USA) along with slide glass, cover glass, mineral oil (Bio-Rad, USA), and tweezers. During the experimental treatment, sebum extracted from the nose was placed on a slide glass, covered with a cover glass, and photographed. ImageJ software was then used to quantify the sebum.

Moisture content evaluation

To evaluate changes in skin moisture content, we applied the Dermalab USB moisture probe (Cortex Technology, Inc., Denmark) and analyzed changes in skin moisture content using the Dermalab USB dedicated software, Application software version 1.09. The DermaLab USB moisture probe operates with a spring mechanism that initiates the measurement process when pressure is applied to the skin, measuring the moisture content in the skin's epidermal layer. The measurement unit is in microsiemens (μS), and a higher measurement value indicates higher skin moisture content.

Before treatment, we collected keratin samples from the right nasal area of both control and experimental group subjects using Keratin stickers. After treatment, we collected keratin samples from the left nasal area and stained them with Coomassie brilliant blue G (Sigma-Aldrich, Inc., USA) for 24 hours.

Assessment of skin roughness

To evaluate changes in skin roughness, we utilized PRIMOS Lite (field of view 45x30 - simple, flexible 3D measuring, GFMesstechnik GmbH, Germany) as shown in Figures 2-9. Three consecutive images of the left nasal area of the test subjects were captured. Measurements with the device were conducted at two time points for both the control and experimental groups: before treatment and after treatment. The captured images were analyzed using PRIMOS dedicated software, PRIMOS Lite software (PRIMOS Lite version 5.6E, Germany). Measurement values were matched to the corresponding measurement areas for analysis. Skin roughness analysis was performed using Star roughness to calculate the variable value Ra (average of all heights and depths to the reference plane). Ra is the most widely used international method for surface roughness measurement, representing the arithmetic average of the highest points and lowest points within the entire measurement range. A lower Ra valueindicates less roughness.

Synergy effect testing

Additionally, to assess any synergy effects, on the last day, we applied the Monteranc pore ampoule to half of the groups and measured the synergy effect on pore area and volume.

Determination of total antioxidant capacity

The determination of the total antioxidant capacity (TAC) in the samples followed the methoddescribed by Prieto et al.[21] This assay relies on the reduction of Mo(VI) to Mo(V) by

the samples, resulting in the formation of a green-colored phosphate/Mo(V) complex under acidic pH conditions. To initiate the assay, 0.5 mL of the samples or standard, with varying concentrations ranging from 12.5 to 150 µg/mL, was mixed with 3 mL of a reaction mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 1% ammonium molybdate within separate test tubes. Subsequently, the test tubes were incubated at 95 °C for 10 minutes to ensure the completion of the reaction. After cooling to room temperature, the absorbance of the samples was measured at 695 nm using a spectrophotometer, with a blank solution serving as the reference. Caffeic acid (CA) was utilized as the standard. The blank solution consisted of 3 mL of the reaction mixture and the corresponding volume of the solvent identical to that used for the samples/standard. This blank was also incubated at 95 °C for 10 minutes, and its absorbance was measured at 695 nm. An increase in the absorbance of the reaction mixture indicated a heightened total antioxidant capacity. Five different concentrations, ranging from 12.5 to 150 µg/mL, were employed for each antioxidant assay. These concentrations were selected through trial and error to encompass a range suitable for representing the rational change in antioxidant activity as the sample concentration increased. This range of concentrations was deemed practical for the subsequent calculation of IC50. Each concentration was tested in triplicate, and the experiment was repeated three times.

RESULTS AND DISCUSSIONS

Pore size

After one week of application, pore size in the butterfly zone, T-zone, and cheek area exhibited the following changes: in the butterfly zone, the average pore size decreased from 0.062 mm² to 0.045 mm², a 27.5% improvement; in the T-zone, it decreased from 0.043 mm² to 0.038 mm², indicating an 11.7% improvement; and in the cheek area, it decreased from 0.054 mm² to 0.042mm², demonstrating a 22.3% improvement (Fig. 1).

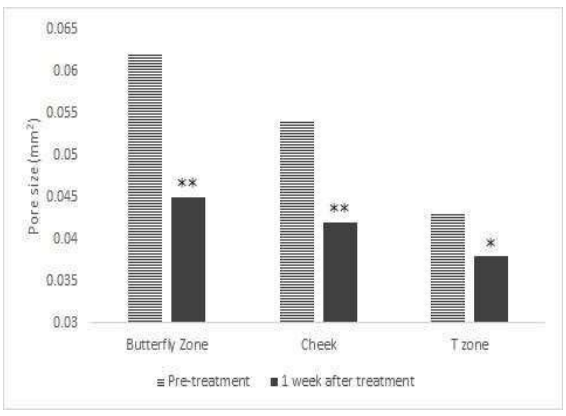

Fig. 1: The changes in pore size in different facial regions. (*: p<0.05, **: p<0.005).

Pore count evaluation

Analysis of pore count using PRIMOS Lite showed that in the control group, there was no change in pore count, remaining at 124.00 pores before treatment and 125.2 pores after treatment. In contrast, the experimental group showed a significant decrease from 124.8 pores before treatment to 67 pores after treatment, representing a 46.3% reduction (Fig. 2). Furthermore, this change was statistically significant when compared to pre-treatment counts ($p<0.005$).

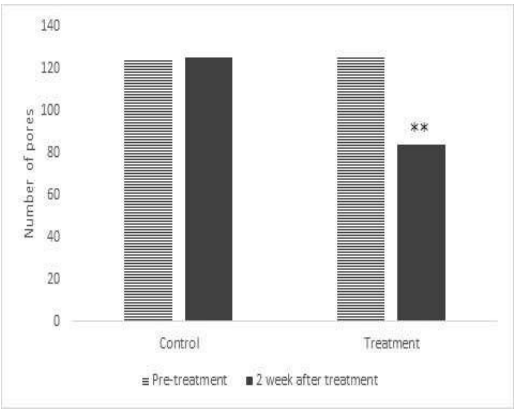

Fig. 2: The changes in pore count two weeks after treatment.

Pore volume assessment

Analysis of pore volume using PRIMOS Lite indicated that in the control group, there was no change in pore volume, remaining at 0.05 μm³ both before and after treatment. Conversely, theexperimental group showed a decrease from 0.055 μm³ before treatment to 0.02 μm³ after treatment, indicating a 63.6% reduction (Fig. 3). This change was also statistically significant when compared to pre-treatment volumes ($p<0.001$).

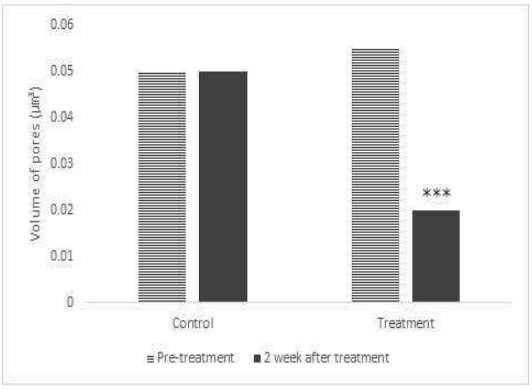

Fig. 3: The changes in pore volume two weeks after treatment.

Sebum assessment

When evaluating sebum using the DermaLab USB sebum probe, the control group showed a

negligible decrease in sebum levels, changing from 7.16 before treatment to 7.08 after treatment. In contrast, the experimental group exhibited a significant reduction, decreasing from 7.23 before treatment to 1.20 after treatment, indicating an 83.5% reduction (Fig. 4). This change was statistically significant ($p<0.001$).

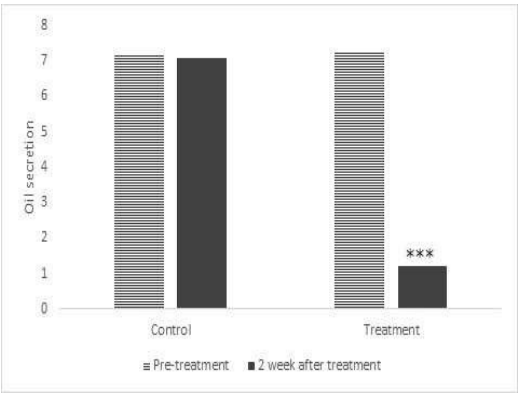

Fig. 4: Evaluation of sebum levels after two weeks.

Moisture content assessment

Analysis of skin moisture content in the nasal area using the DermaLab USB moisture probe showed that in the control group, there was no significant change, with levels going from 582.39 μS to 584.32 μS. In contrast, the experimental group demonstrated a substantial increase, rising from 585.51 μS before treatment to 1080.83 μS after treatment, indicating a 184.5% increased (Fig. 5). This change was statistically significant ($p<0.001$).

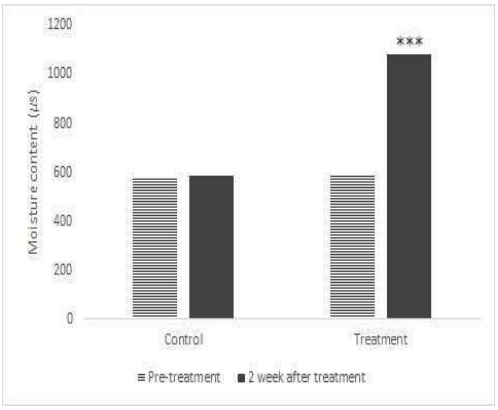

Fig. 5: Evaluation of moisture content levels after two weeks.

Sebum and Blackhead evaluation

Analysis of blackhead pore count using the full-face imaging system showed that in the control group, there was no significant change in blackhead pore count or sebum levels before and after treatment. However, in the experimental group, it was visually confirmed that the use of the cosmetic composition resulted in a reduction in blackheads and a higher amount of

sebum being exposed on the skin surface when compared to before treatment. After two weeks, sebum wasremoved using the rolling method, and the amount of removed sebum was measured. The results showed that a total of 20,305 sebum plugs were removed in the control group, while in the experimental group, a total of 164,316 sebum plugs were removed, indicating an 809% higher sebum removal rate in the experimental group compared to the control group (Fig. 6).

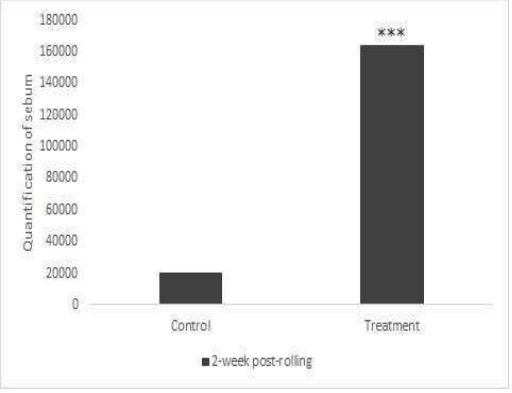

Fig. 6: The difference in sebum removed by the rolling method. (*: p<0.001).**

Keratin level assessment

Analysis of the skin keratin improvement score using Keratin stickers and a Digital light microscope revealed that in the control group, the average score decreased from 4.04 before treatment to 4.00 after treatment, representing a 0.99% improvement. In contrast, the experimental group showed a significant change, with the score decreasing from 4.00 before treatment to 2.35 after treatment, indicating a 41.25% improvement in keratin levels followingthe application of the test material (Fig. 7). This change was statistically significant.

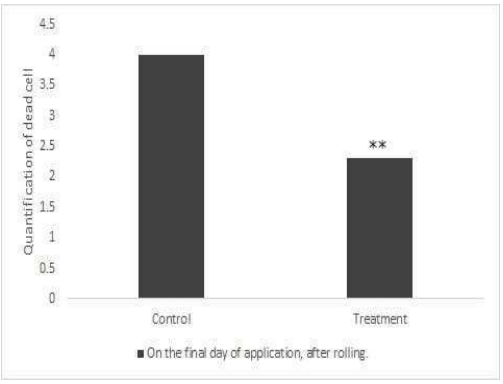

Fig. 7: The difference in remaining keratin levels after removal by the rolling method. (: p<0.005).**

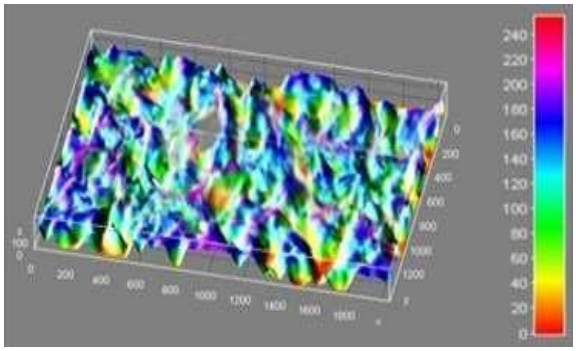

Fig. 8)

Skin roughness evaluation results

Analysis of skin roughness values using PRIMOS Lite revealed that in the control group, there was a statistically significant improvement in skin roughness, with the pre-treatment value decreasing from 20.03 to 19.98 after two weeks (Fig. 9). In the treatment group, the pre- treatment value was 20.02, and it significantly decreased to 13.45 after two weeks ($p<0.005$).

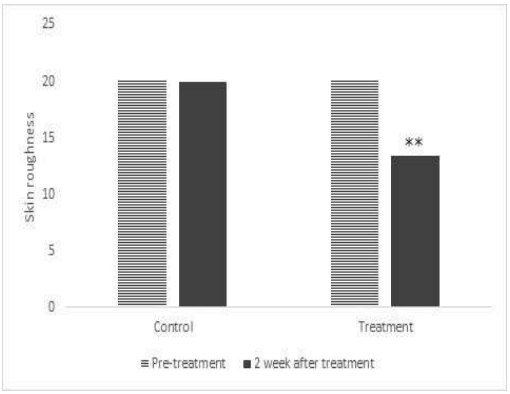

Fig. 9)

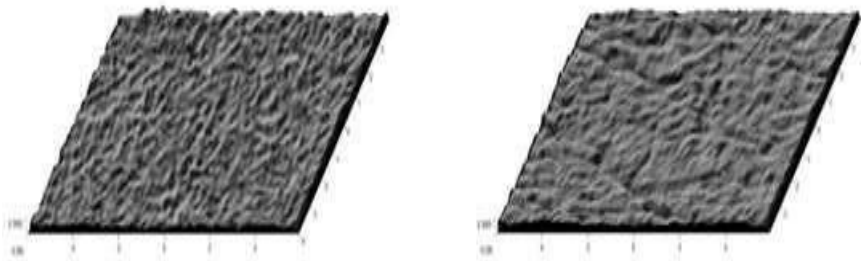

Fig. 10)

Total antioxidant capacity

These solutions exhibited substantial antioxidant activity when compared to the standard Caffeic Acid (CA). At a concentration of 100 µg/mL, the TAC values ranged from 0.510 ± 0.025to 1.84 ± 0.090. Similarly, at 150 µg/mL, the TAC values were in the range of 0.635 ±

0.028 to 2.300 ± 0.045. As the concentration of the extracts increased, the overall antioxidant activity exhibited an upward trend.

CONCLUSIONS

In summary, we observed a reduction in pore size, a decrease in pore count, a decrease in pore volume, a reduction in oil content, sebum levels, and an increase in moisture content when using Monteranc Baby Pore Essence. Moreover, it was confirmed that Monteranc Baby Pore Essence exhibited significant antioxidant activity. Additionally, the application of the rolling method once resulted in the significant removal of sebum and keratin, leading to an improvement in skin texture and complexion.