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CENTRE FOR SCIENCE AND RESEARCH

Combination of pre-, postbiotic and *Humulus lupulus* extract complex on skin microbiome

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INTRODUCTION

Skin microbiota is known to be a crucial factor of proper skin homeostasis, especially in diverse skin dermatoses. The aim of this study was to evaluate the impact of three cosmetic ingredients: prebiotic (inulin and alpha-oligossacharide), postbiotic (*Lactobacillus* ferment lysate) and *Humulus lupulus* extract (as complex **SENSIBIOME**) on keratinocytes viability in presence of *S.epidermidis* or *S.aureus* mixture, and formation of these bacteria biofilm *in vitro*. Influence of skin condition of SENSIBIOME in topical formulation were also assessed.

MATERIALS AND METHODS

- *In vitro* viability of HaCaT in presence of *S.aureus* ATCC 6538 and *S.epidermidis* ATCC 14990 in monoculture and different concentrations of single ingredients or SENSIBIOME, was visualized by SYTO/PI staining. Biofilm of bacteria strains was also evaluated after single ingredient treatment as well as their complexes.
- *In vivo* evaluation of **serum no. 16013** containing SENSIBIOME (inulin and alpha-oligossacharide 0,1%; *Lactobacillus* ferment lysate 0,2%; *Humulus lupulus* extract 0,1%) was performed on a group of 24 adult volunteers with sensitive and allergy prone skin for 3 weeks. One person terminated the test due to skin discomfort. Skin parameters before and after test were evaluated: erythema (Mexameter) and red areas (Visia) related to sensitive skin visualisation. Patients were evaluating their skin condition before and after serum usage according to SensiScale-10 survey[1].

RESULTS

In vitro results showed diverse keratinocytes viability in presence of bacteria strains and single ingredients. All concentrations of tested ingredients showed increse in *S.auresus* biofilm formation with decrese in *S.epidermidis* biofilm. Completely opposide results were achieved while different concentrations of **SENSIBIOME** were used, suggesting the positive effect of all three ingredients combined together on skin microbiota homeosthasis. In the *in vivo* test, the reduction of erythema was observed. Moreover, reduction of sensitive skin symptoms by 55% evaluated in SensiScale-10 was demonstrated.

Cell viability *in vitro*

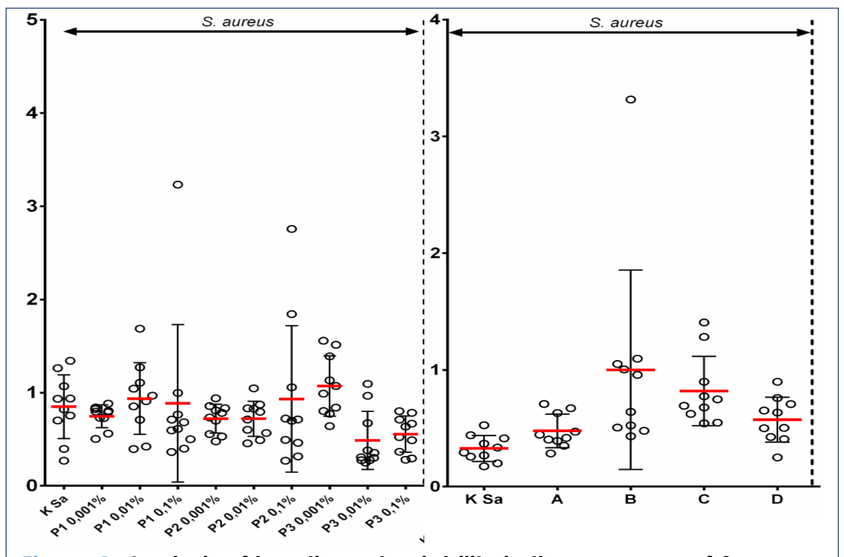


Figure 1. Analysis of keratinocyte viability in the presence of *S. aureus*. Results of analyses for individual substances – ratio of SYTO 9 signal intensity to propidium iodide. The symbols represent the mean values for keratinocytes from the entire single field of view; the red dash is the average value for all analyzed fields; the whiskers represent the standard deviation. Empty symbols refer to keratinocytes treated with bacterial solutions. K Sa = HaCaT cells + *S. aureus*. **For the single ingredients, the improvement in cells' viability in bacterial presence, was not detected, except of P3. For all complexes slight increase of cells' viability was observed.**

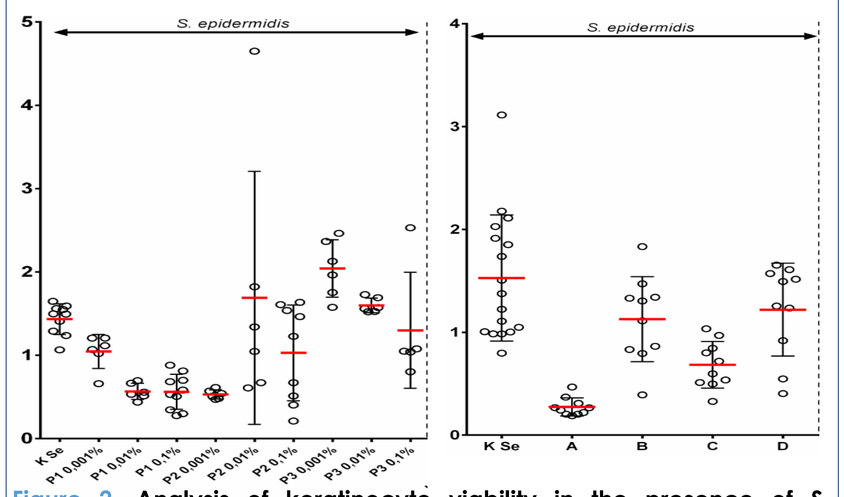


Figure 2. Analysis of keratinocyte viability in the presence of *S. epidermidis*. Results of analyses for individual substances – ratio of SYTO 9 signal intensity to propidium iodide. The symbols represent the mean values for keratinocytes from the entire single field of view; the red dash is the average value for all analyzed fields; the whiskers represent the standard deviation. Empty symbols refer to keratinocytes treated with bacterial preparations. K Se = HaCaT cells + *S. Epidermidis*. **For the single ingredients, the improvement in cells' viability in bacterial presence was observed for P3. Moreover, for all concentrations of P1 there was inhibition of keratinocytes viability. However for all complexes the reduction of cells's viability were obtained.**

Biofilm formation *in vitro*

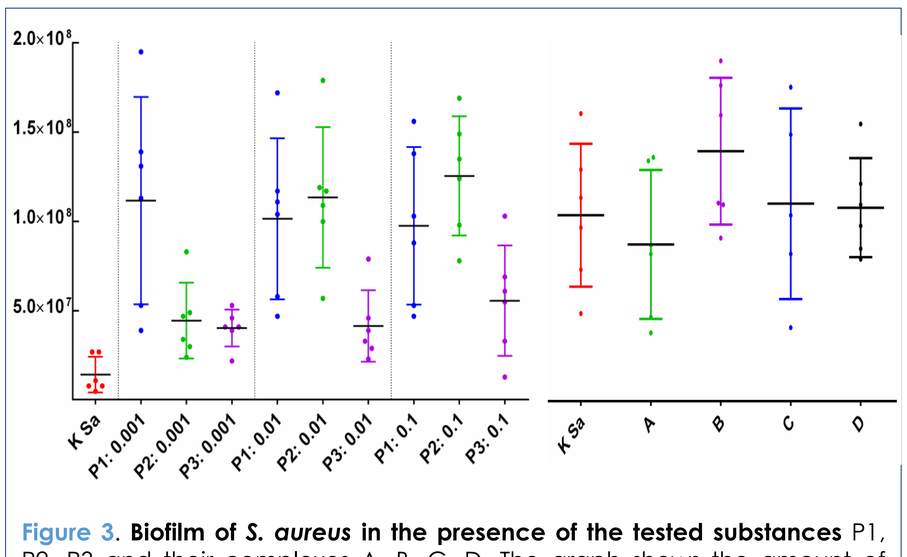


Figure 3. Biofilm of *S. aureus* in the presence of the tested substances P1, P2, P3 and their complexes A, B, C, D. The graph shows the amount of biofilm formed, obtained in a range of dilution (points), the average of the results obtained (black line) and the standard deviation (whiskers). **All samples at each concentrations showed a statistically significant increase in *S. aureus* biofilm formation compared to the control. Unexpectedly, the combination of substances in the all tested complexes did not affect the growth of *S. aureus* biofilm, which was observed when using single substances.** In the case of complex A, a slight inhibition of biofilm formation was observed, but it is not statistically significant compared to the control. The complex B slightly increases the amount of *S. aureus* biofilm, but not statistically significantly (p>0.05).

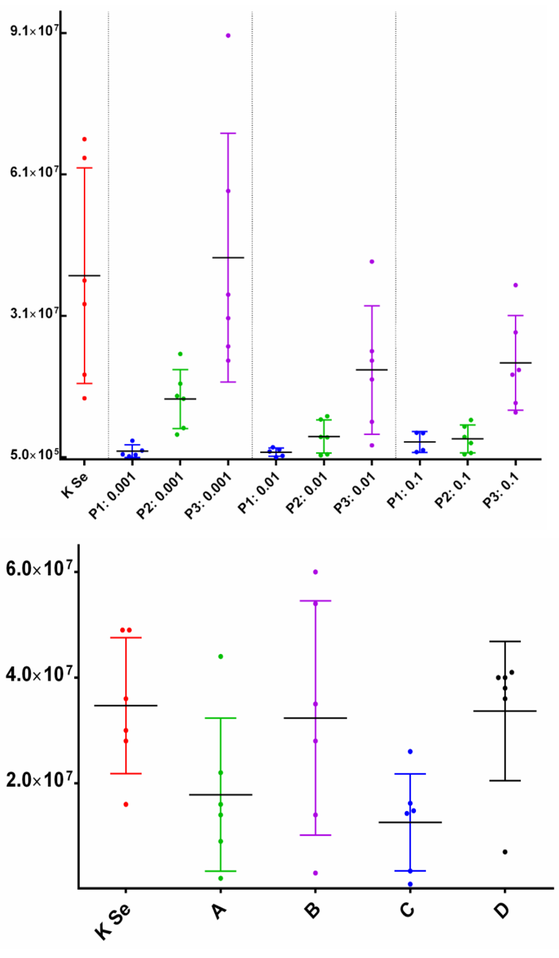


Figure 4. Biofilm of *S. epidermidis* in the presence of P1, P2, P3 single substances (upper panel) and their complexes A, B, C, D (lower panel). The graph shows the amount of biofilm formed, obtained in several dilution (points), the average of the results obtained (black line) and the standard deviation (whiskers). **P1 has an inhibitory effect on the formation of *S. epidermidis* biofilm ($p<0.05$) at all concentration used. P2 also has an inhibitory effect, lower than P1, but still statistically significant ($p<0.05$). P3 does not show a statistically significant difference in biofilm inhibition ($p<0.05$), but the average biofilm amounts obtained at concentrations of 0.1% and 0.01% are lower than in the control. Complexes A and C showed a statistically significant ability to inhibit the formation of *S. epidermidis* biofilm, while complexes B and D do not have such an effect compared to the control sample.**

Legend of ingredients and solutions: **P1** = inulin and alpha-oligossacharide; **P2** = *Lactobacillus* ferment lysate; **P3** = *Humulus lupulus* extract; K Sa = HaCaT cells + *S. aureus* bacteria; **A** = P1 0,1%, P2 0,001%, P3 0,001%; **B** = P1 0,01%, P2 0,001%, P3 0,001%; **C** = P1 0,1%, P2 0,001%, P3 0,01%; **D** = P1 0,1%, P2 0,01%, P3 0,1%

Evaluation of skin condition

Table 1. **Skin sensitivity evaluated by patients according to SensiScale-10**[1]. Results demonstrate reduction in skin sensitivity sympthoms in average by 55%.

Degree of overall skin irritation during the past 3 days before and at the end of the test (n=23); score >13 - sensitive skin			
D0	**D21**	**difference**	**% of reduction**
23,95	10,73	-13,22	55%

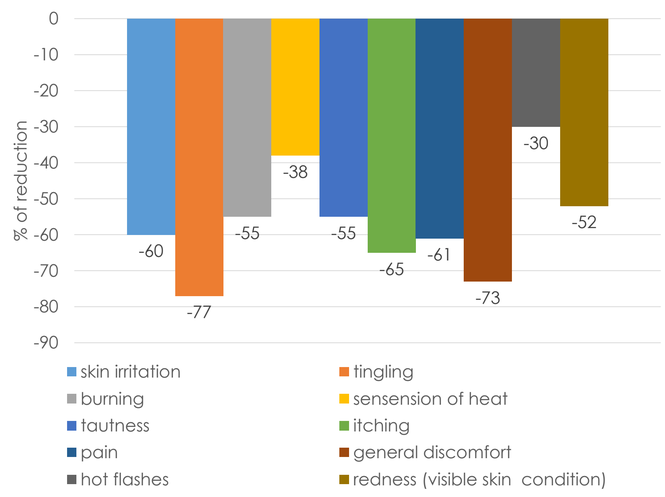
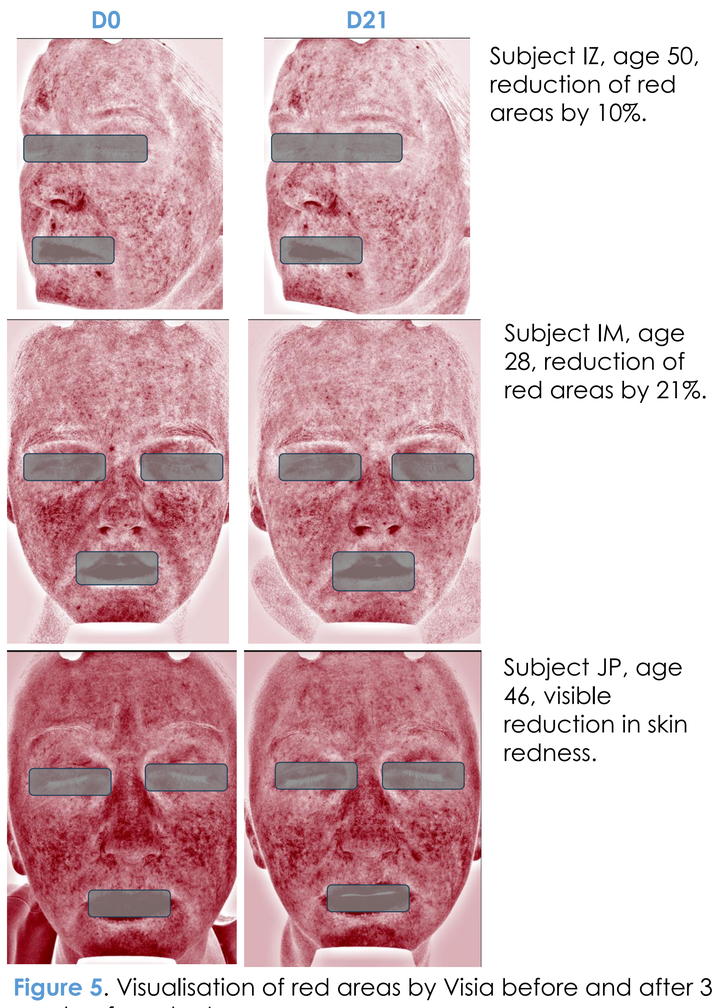


Table 2. **Instrumental evaluation of skin condition before and after 3 weeks of product usage**. Erythema measured by Courage&Khazaka probes; Red areas measured by Visia.

Instrumental evaluation of erythema (n=11)	
Skin parameter	**Results**
Erythema	Reduction by 9% in 64% of patients (Mexameter) and for whole group reduction by 5% (VISIA)



Subject IZ, age 50, reduction of red areas by 10%.

Subject IM, age 28, reduction of red areas by 21%.

Subject JP, age 46, visible reduction in skin redness.

Figure 5. Visualisation of red areas by Visia before and after 3 weeks of product usage.

CONCLUSION

Combination of pre-, postbiotic and anti-inflammatory *Humulus lupulus* extract display calming and soothing properties via rebalancing skin microbiota. According to *in vitro* study the best results were obtained for complexes B and D where the slight increase of skin cells viability was observed in presence of *S. aureus* with simultanius maintance of *S. epidermidis* growth.

LITERATURE

1. Misery L. et al. J Eur Acad Dermatol Venereol. 2016 Feb:30 Suppl 1:2-8.