51th Annual ESDR Meeting 2022, Amsterdam, Netherlands

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Targeting *Staphylococcus aureus* biofilm with leave-on emollients containing oligofructants and acetyl heptapeptide-4 complex

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INTRODUCTION

Increased Staphylococcus aureus colonisation is associated with atopic eczema (AE) severity. Reduction of S. aureus levels, especially in AD patients, may led to an improvement in the skin condition. **Thus, the aim of a study was to evaluate the reduction od S. aureus biofilm formation, of two emollients for face and body care (no. 19624 and 19625 respectively).** They contained complex of two ingredients (oligofructants from *Ophiopogon japonicus* and acetyl heptapeptide-4). Moreover safety and tolerability of mentioned products were evaluated.

MATERIALS AND METHODS

- In plates, bacterial suspensions of strains *Staphylococcus aureus* MSSA (83254), MRSA (BH1CC), ATCC 6538 and three strains of *S. epidermidis* (RP62A, 1457 and 12228) were mixed and incubated with several dilutions of tested emollients for 24 hrs, stained with crystal violet.
- MTT cytotoxicity in vitro (L929 cells) and irritation potential ex vivo on EpiDerm skin model were measured according to ISO 10993.
- In addition to this, the severity of AD and occurence of flares-up in children was tested in a preliminary studies in comparison to topical medical treatement.

RESULTS

Safety study *in vitro*

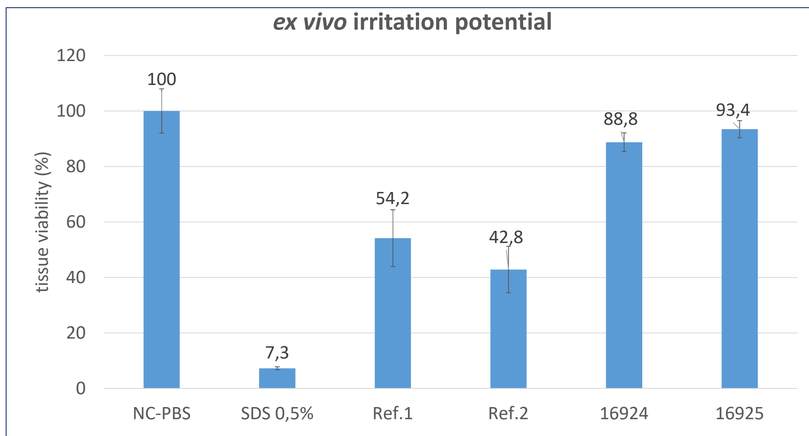

Figure 1. Skin irritation potential of tested products on EpiDerm model. Ref 1- naphthalene acetic acid (CAS 86-87-3) – non classified (non irritant). Ref 2 - cyclamen aldehyde (CAS 103-95-7) – classified (irritant, Cat. 2). Correlation of *in vitro* and *in vivo* results: Tissue viability ≤ 50% of the control (PBS) — irritant (R38). Tissue viability ≥ 50% of the control — non-irritant.

The tested products were confirmed as non-irritant on EpiDerm skin model, resulting in the mean tissue viability of 88,8% (for face cream 16924) and 93,4% (for body balm 16925).

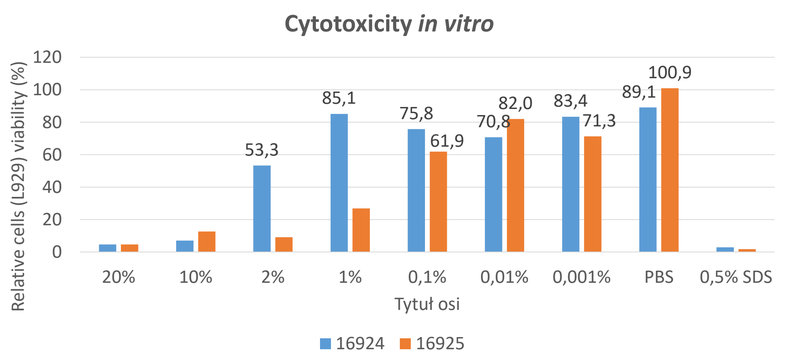

Figure 2. Cytotoxicity of tested products on L929 cells. Viability <70% of the control - cytotoxic potential. Ref – 0,5% SDS. **The tested products were non-cytotoxic at the concentration at least or equal to 1% (cells viability: 85,1% for face cream 16924) and 0,01% (cells viability: 82% for body balm 16925).**

Adhesion and biofilm formation *in vitro*

Reduction of biofilm after exposition to tested products, as % reduction of absorbance A_{595}	Reduction A_{595} (%) ± SD	
	S.aureus 6538	*S.epidermidis* 12228
Product no. 16924	82,2± 4,5	78,3 ± 1,7
Product no. 16925	30,6± 4,2	- 138,2 ± 57,3 (stymulation of biofilm formation)

Table 1. Reduction of biofilm formation of *S. aureus* and *S. epidermidis* growing in medium containing several concentrations of tested products.

Dose-dependent effect on *Staphylococcus spp.* on adhesion and biofilm formation *in vitro*

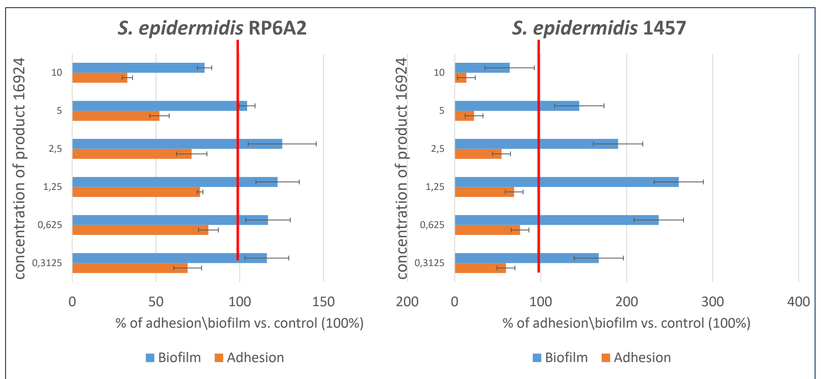

Figure 3. Biomas level in % due to adhesion or biofilm formation by *S. epidermidis* strains 1457 and RP62A growing in medium containing different concentrations of face cream no 16924. **Product reduces adhesion of *S. epidermidis* to abiotic surfaces.** Result is directly propotional to concentration of product used in tests. **Biofilm formation was stimulated in most face cream tested concentrations.**

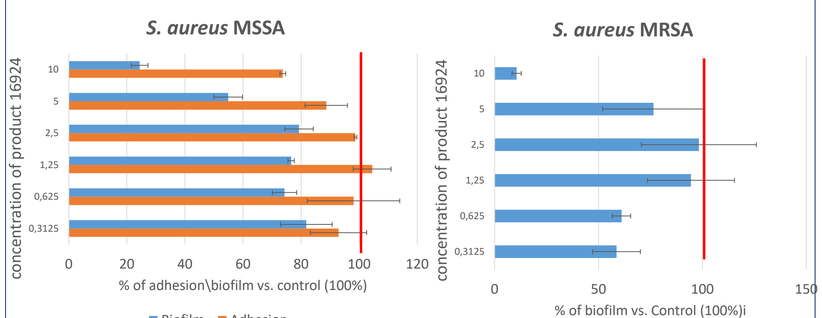

Figure 4. Biomas level in % due to adhesion or biofilm formation by *S. aureus* strains MRSA and MSSA growing in medium containing different concentrations of face cream no. 16924. **For MSSA strain inhibition of adhesion was in concentrations 2,5-10% and biofilm formation was reduced in all cases. For MRSA strain the inhibition of biofilm formation was observed in concentrations of product 0,3125-0,625% and 5-10%.**

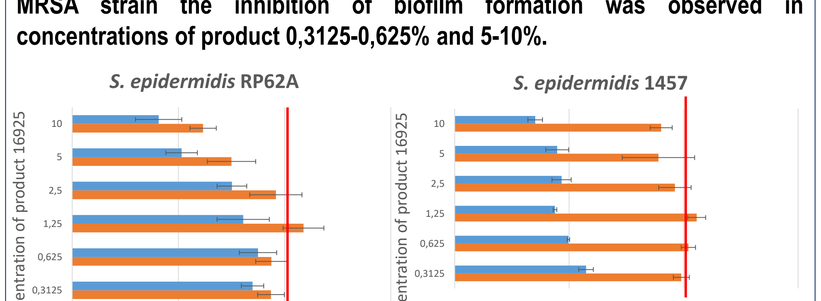

Figure 5. Biomas level in % due to adhesion or biofilm formation by *S. epidermidis* strains 1457 and RP62A growing in medium containing different concentrations of body balm no 16925. **Product reduces adhesion of *S. epidermidis* RP62A to abiotic surfaces and reduces formation of biofilm.** Adhesion was reduced in higher concentrations of cream above 2,5%. In strain *S. epidermidis* 1457 no significant changes in adhesion could be observed. Only inhibition of biofilm was observed.

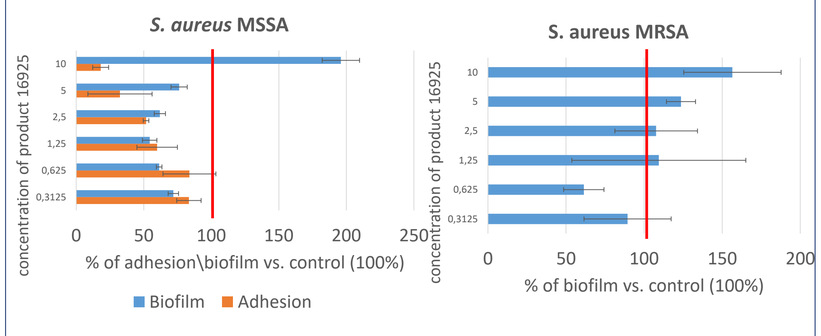

Figure 6. Biomas level in % due to adhesion or biofilm formation by *S. aureus* strains MRSA and MSSA growing in medium containing different concentrations of body balm no. 16925. **For MSSA strain inhibition of biofilm formation was in concentrations 0,3125-1,25% but for adhesion is direct propotional result.** For MRSA strain the inhibition of biofilm is only visible in 0,625% concentration of product. For the rest of concentrations the stymulation of growth could be detected.

Clinical evaluation of skin condition

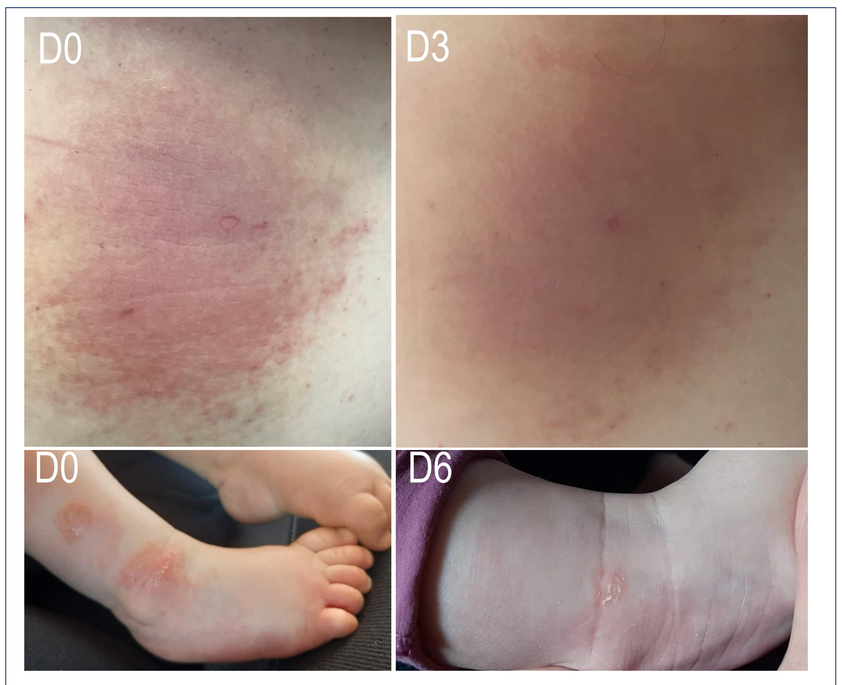

Figure 7. Both patients were used body balm 16925. Upper photo: Adult, female patient. Visible flare-ups on abdomen. **Reduction of flare in 3 days after product 16925 usage was noticed.** Lower photo: Newborn patient with flare on the leg and anckle. **Reduction of flare visibility after 6 days of product 16925 usage was also observed**. No other treatment was used in both cases.

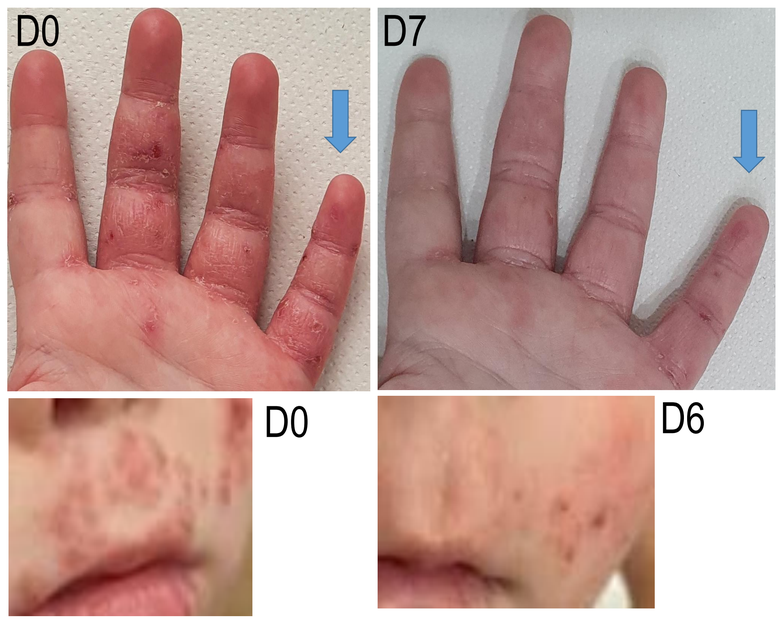

Figure 8. Upper photo: Patient ID 1 (female, 8 yrs). Visible flare-ups on hands. Fifth finger of the left hand was treated with product no. 16925 (narrow), the rest was treated with drug formulation gentamycini 0,04; hydrocortisoni 1,0; ung. Cholesteroli 100,0. **The efficacy of product no. 16925 was comparable to medical treatement.**
The lower photo: Patient ID 5A (famele, 12 months). **Reduction of flare visibility after 6 days of face cream 16924 usage was observed.** No other treatement was used.

Clinical evaluation of skin condition in analogue scale		
Sympthom	Face cream 16924, n= 4 [% of improvement}	Body balm 16925, n=8 [% of improvement]
Skin redness	77,8	75,0
Edema/papules	62,5	69,0
Oozing/crust	33,3	64,6
Excoration	75,0	68,5
Lichenification	66,7	69,0
Itch	68,4	52,2
Sleep disturbance	62,5	60,8

Table 2. Clinical evaluation of skin condition in 4-point analogue scale (3-severe symptoms, 0-lack of symptoms) and subjective opinion about itch and sleep disturbane in 10-point analogue scale (10-severe, 0-lack of symptoms) of product no. 16924 and 16925. **The improvement in all evaluated sympthmoms was observed**.

CONCLUSION

- Both products were biocompatible with the skin and well-tolerated.
- The influence of product on biofilm formation was dependent from bacterial strain used in tests.
- Both products may be considered as an effective Emollient Plus type of devices to reduce flares-up in patients suffering for atopic eczema.
- Both products can support medical treatment or be used as first choice therapy in AE.