Active ingredient from *Bombax costatum* (kapok tree) to preserve skin microbiota equilibrium

Leclère-Bienfait, Sophie[1*]; Brédif, Stéphanie[1]; Boyer, Gaëtan[1]; Le Roux, Marion[1]; Bellemère, Gaëlle[1]; Baudouin, Caroline[1]

[1] Innovation Research and Development, Laboratoires Expanscience, Epernon, France

* Sophie Leclère-Bienfait +(33)2 37 20 33 33, sleclere@expanscience.com

Introduction.

Skin is colonized by a wide variety of microbes. Preserving skin microbiota homeostasis is of crucial importance for maintaining healthy skin. We have developed a new active ingredient from *Bombax costatum* flowers. This polysaccharide-rich extract was evaluated *in vitro* on different microbiota models showing its ability to preserve skin microbiota equilibrium.
A clinical study has been performed showing its capacity to maintain the intimate area of women in good condition and to rebalance the intimate microflora.

Materials and Methods.

In vitro evaluation

Bacterial strains:
Staphylococcus aureus; *Staphylococcus epidermidis*; *Cutibacterium acnes; Staphylococcus hominis; Lactobacillus gasseri, L. acidophilus, L. rhamnosus, L. crispatus et L. jensenii.*

Bacterial growth study:
A mixture of different bacterial strains, representative of cutaneous microbiota, were cultivated in a liquid medium for 48 hours in presence of the extract. The quantity of the inoculated bacteria was determined in order to get either balanced proportions of each strain or with decreased ratio of S. aureus compared to other strains (ratios 1:1, 1:100 or 1:1000). The growth of each bacterium was evaluated after subculture on specific agar.

Adhesion study:
Reconstructed human epidermis (RHE) were topically treated by the extract prior to the deposition of *S. aureus* or *S. epidermidis* solutions. After incubation, bacteria adhering to the RHE were quantified by colony forming unit counting after seeding on specific agar.

Biofilm study:
Biofilm formation of *S. aureus* and *S. epidermidis* was studied using crystal violet dye in multiwell plate. An aliquote of bacterial culture was deposited and incubated in presence of the extract for 24h.At the end of static incubation, the biofilm was stained using crystal violet and quantified by optical density measurement.

Epidermal response studies:
Normal human keratinocytes were incubated for 48h in presence of the extract. The expression level of innate immunity markers TLR2 (Toll-Like Receptor 2) and hBD2 (human beta-Defensin 2) were evaluated by ELISA assay.
RHE, topically pre-treated for 24h by the extract, were incubated in presence of *S. aureus* secretum for 24h. Histological analysis were performed after barrier markers immunostaining.

Lactobacilli prebiotic effect study:
Lactobacilli strains, representative of vaginal microflora, were cultivated in a liquid medium for 4 or 24 hours in presence of the extract. The growth of each bacterium was evaluated after subculture on specific agar. The acid lactic production was evaluated in culture medium after 24 hours.

In vivo evaluation

A clinical study under gynecological control has been performed to evaluate the capacity of *Bombax costatum* extract to maintain the intimate area of women in good condition and to rebalance the intimate microflora which could be affected after vaginosis or mycosis pathologies, in case of irritations or in menopaused women.
4 groups of subjects used an active cream combined with active cleansing gel containing both 1.25% of *Bombax costatum* extract. For each group inclusion criteria were:

- Groupe 1: “Post-Vaginosis”: subjects between 18 and 50 years old and in post treatment for bacterial vaginosis (no symptoms present at inclusion). (n=11 subjects)
- Groupe 2: “Post-Mycosis”: subjects between 18 and 50 years old and in post treatment for mycosis (no symptoms present at inclusion). (n=12 subjects)
- Groupe 3: “Irritation”: subjects over 18 years old presenting vulvar redness / irritation and vaginal discomfort sensations without clinically proven pathology. (n=13 subjects)
- Groupe 4: “Menopaused” Active: subjects over 50 years old in post menopause and complaining of a feeling of vulvar and vaginal dryness. (n=12 subjects)

A control group which used cream and cleansing gel without *Bombax costatum* extract was also include for comparison with group 4:

- Groupe 5: “Menopaused” Control: subjects over 50 years old in post menopause and complaining of a feeling of vulvar and vaginal dryness. (n=12 subjects)

Subjects used cream twice a day, after a hygiene routine or at any time of day with massage until fully absorbed and cleansing gel once a day as a liquid soap on the intimate area (vulvar area including vestibule and perineum and perianal area) for 28 days.
Evaluations included:

- Clinical scoring of erythema, dryness, fissures, global irritation (10 points scale).
- Auto-scoring of burning sensations, pruritus and dyspareunia (10 points scale).
- pH measurement.
- Vulvar sampling using swabs for inflammation biomarker (IL1-α cytokines) and micro-flora (total flora, *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus jensenii and Lactobacillus gasseri*) assessment.

Lactobacillus bacteria were chosen according to literature resources describing them as representative of intimate area health [1].
Groups which used active cream and cleansing were pooled for analysis, and comparison between group 4 and 5 was performed.

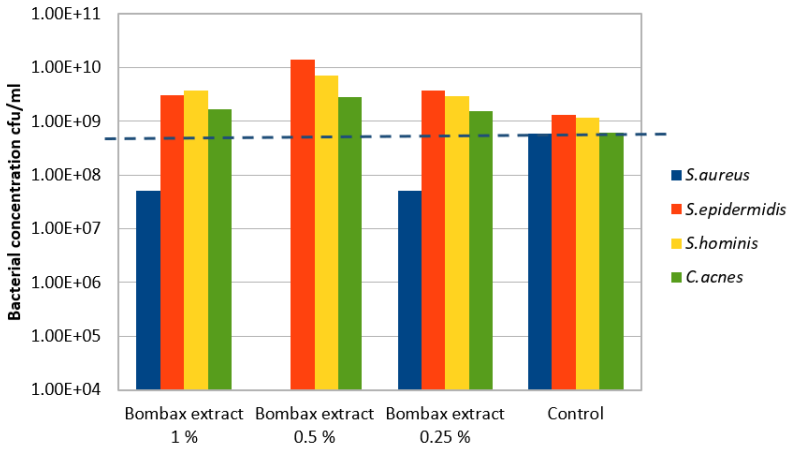
Results.

In vitro evaluation

Regulation of bacterial growth
The *Bombax costatum* polysaccharide-rich extract tended to inhibit the growth of the pathogenic strain *S. aureus*, without significantly affecting the growth of commensal bacteria (*S. epidermidis, S. hominis, C. acnes*) (Fig.1). This effect was even more significant as *S. aureus* was present in high

proportions compared to other strains (data not shown), suggesting a preventive effect toward *S. aureus* pathogenicity by limiting its overgrowth.

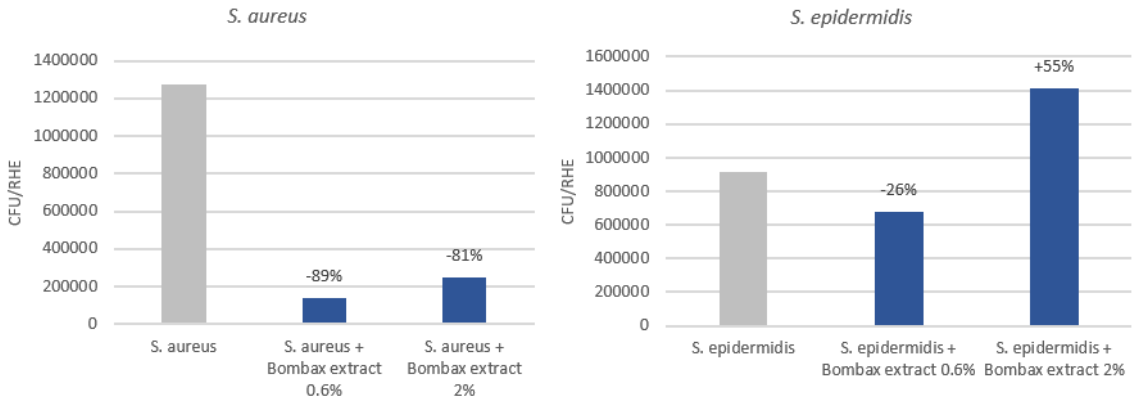
Figure 1: Bacterial growth after 48h - Ratio 1:1.



Regulation of bacterial adhesion

The extract was able to deeply decrease *S. aureus* adhesion on RHE without importantly affecting *S. epidermis* adhesion (Fig. 2).

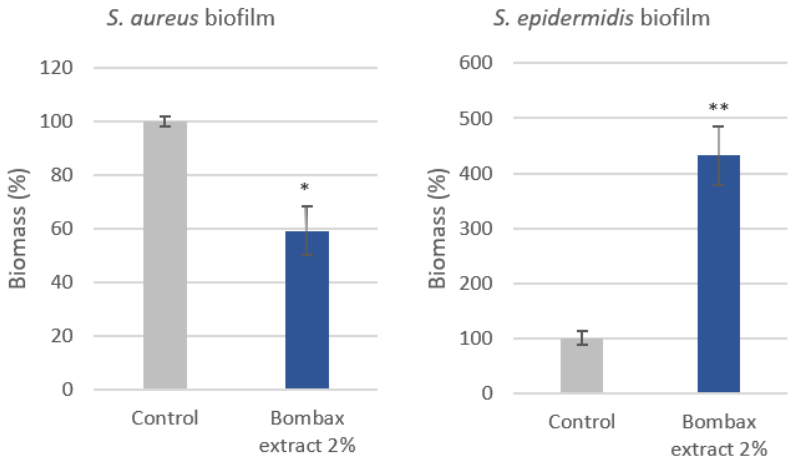
Figure 2: *S. aureus* and *S. epidermidis* adhesion on RHE.



Regulation of biofilm formation

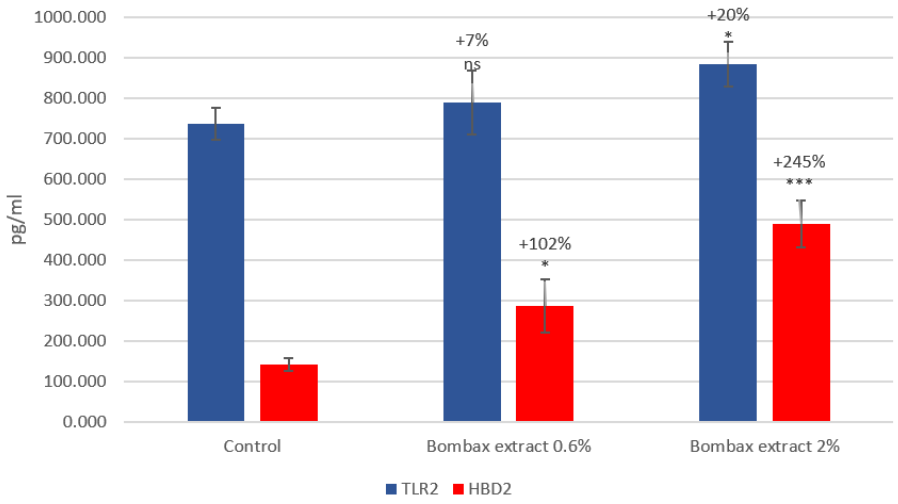
The extract significantly inhibited *S. aureus* biofilm formation when it increased that of *S. epidermidis* (Fig. 3).

Figure 3: Evaluation of biofilm formation after 24h incubation.



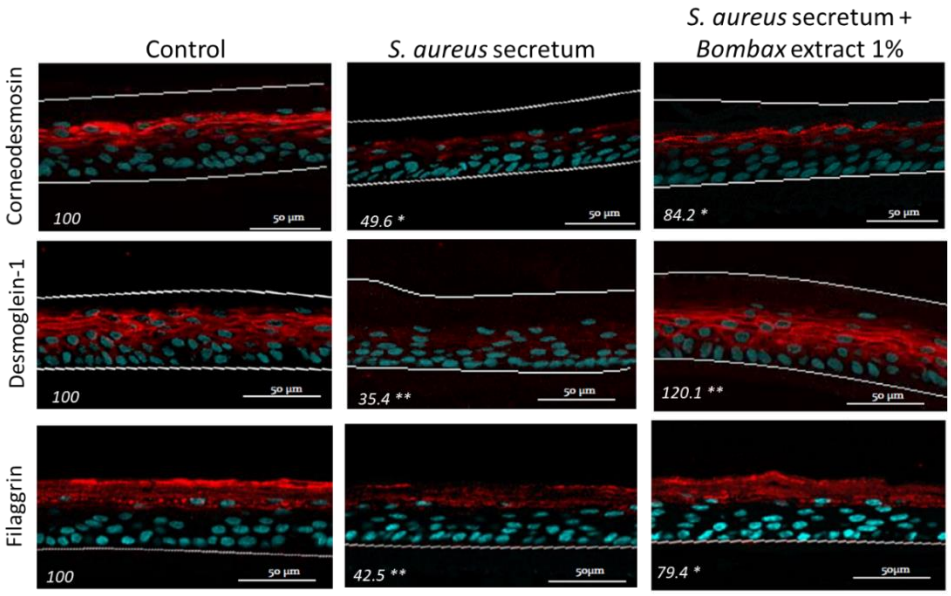
Stimulation of epidermal innate immune response
The extract significantly increased keratinocyte production of TLR2 and anti-microbial peptide hBD2 (Fig. 4).

Figure 4: TLR2 and hBD2 production by NHEK.



Protection against S. aureus-induced damages to epidermal barrier
The application of *S. aureus* secretum on reconstructed epidermis induced an alteration of epidermal barrier markers (Fig.5). The *bombax* extract, topically applied prior to *S. aureus* secretum, was able to preserve the expression of filaggrin, desmoglein-1 and corneodesmosin, thus showing a protective effect of barrier function.

Figure 5: Fluorescent immunostaining of barrier markers in RHE.
Quantification by image analysis. * p<0.05; ** p<0.01 - Unpaired t test.



Vaginal microflora equilibrium

The *bombax* extract showed a positive effect on the growth of lactobacilli (*L. gasseri, L. acidophilus, L. rhamnosus, L. crispatus et L. jensenii).* These results show a prebiotic effect of the active ingredient against these strains (Table 1). The extract, also, inhibited the growth of two pathogen strains *(G. vaginalis et E. coli),* showing a bacteriostatic or even antimicrobial effect (Table 1).

The *bombax* extract increased the lactic acid production by *L. acidophilus* promoting the lactobacillus metabolism (Table 2). Lactic acid is important to maintain an acid pH in this area which is essential for maintaining a healthy vaginal microflora.

Table 1: Evaluation of viability of vaginal bacteria
*** p<0,05 ; ** p<0,01 vs Control**
One way ANOVA followed by Tukey test.

	L. gasseri *(Log_{10} CFU/ml)*		***L. acidophilus*** *(Log_{10} CFU/ml)*		***L. rhamnosus*** *(Log_{10} CFU/ml)*	
	4h	*24h*	*4h*	*24h*	*4h*	*24h*
Control	7,98 ± 0,01	3,10 ± 0,28	7,77 ± 0,13	5,84 ± 0,09	7,82 ± 0,21	8,23 ± 0,00
Glucose 2%	7,81 ± 0,27	3,42 ± 0,14	7,73 ± 0,23	7,22 ± 0,15	7,83 ± 0,06	9,09 ± 0,07
BCP 0,125%	7,89 ± 0,14	2,49 ± 0,12*	7,72 ± 0,13	**8,99 ± 0,55***	7,77 ± 0,10	8,89 ± 0,27
BCP 0,25%	8,01 ± 0,19	0,00 ± 0,00**	7,74 ± 0,08	6,10 ± 0,57	7,83 ± 0,06	8,51 ± 0,56
BCP 0.5%	8,02 ± 0,10	3,27 ± 0,38	8,10 ± 0,58	6,29 ± 0,02	7,74 ± 0,06	7,82 ± 0,01*
BCP 1%	**10,40 ± 0,24****	3,73 ± 0,07	8,83 ± 0,18	6,63 ± 0,25	**8,75 ± 0,11****	**10,35 ± 0,04***

	L. crispatus *(Log_{10} CFU/ml)*		***L. jensenii*** *(Log_{10} CFU/ml)*		***G. vaginalis*** *(Log_{10} CFU/ml)*		***E. coli*** *(Log_{10} CFU/ml)*	
	4h	*24h*	*4h*	*24h*	*4h*	*24h*	*4h*	*24h*
Control	7.84	7.29	7.23	8.28	8.7	8.87	8.98	8.64
Glucose 2%	9.24	8.29	9.23	8.84	9.4	9.32	8.46	8.78
Ciprofloxacine	-	-	-	-	5.47	4.71	5.39	2.56
BCP 0,25%	8.64	**8.26***	**8.55***	8.47	8.99	**7.67****	**8.02****	8.33
BCP 1%	8.79	7.78	**9.01****	8.97	**7.19***	**7.04****	**7.88***	**7.25****

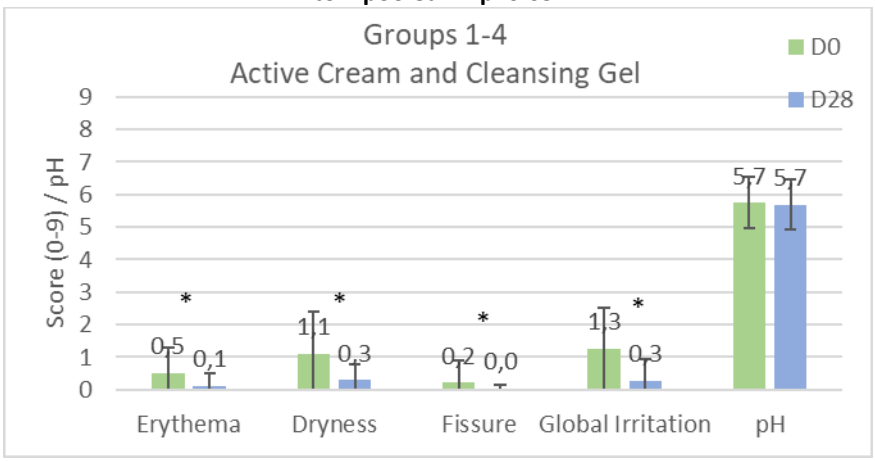
Table 2: Lactic acid production by *L. acidophilus.*

	Lactic acid *(nmol/µL)*	*Evolution*
Control	25421.81 ± 4389.11	
Glucose 2%	24200.96 ± 1062.12	-5%
BCP 0,125%	**57556.58 ± 29969.59**	**+126%**
BCP 0,25%	**46584.36 ± 2749.86**	**+83%**
BCP 0,5%	**48314.47 ± 12267.67**	**+90%**
BCP 1%	**90768.17 ± 945.72**	**+257%**

In vivo evaluation

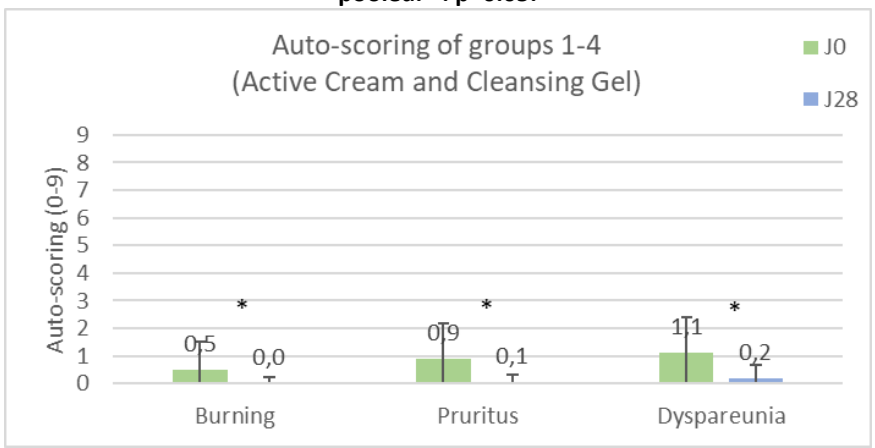
Bombax costatum extract significantly improved erythema, dryness, fissure and global irritation assessed by clinical scoring as illustrated figure 6. pH was not disturbed and stayed in normal physiological range.
Limit statistically difference (p<0.1) in favor of *Bombax costatum* extract was observed between group 4 and 5 for the dryness.

Figure 6: Clinical scoring of erythema, dryness, fissure, global irritation, and pH at D0 and D28 for groups 1 to 4 pooled. *: p<0.05.



Bombax costatum extract significantly improved burning sensation, pruritus and dyspareunia expressed by the subjects themself as illustrated figure 7.

Figure 7: auto-scoring of burning, pruritus and dyspareunia sensations at D0 and D28 for groups 1 to 4 pooled. *: p<0.05.

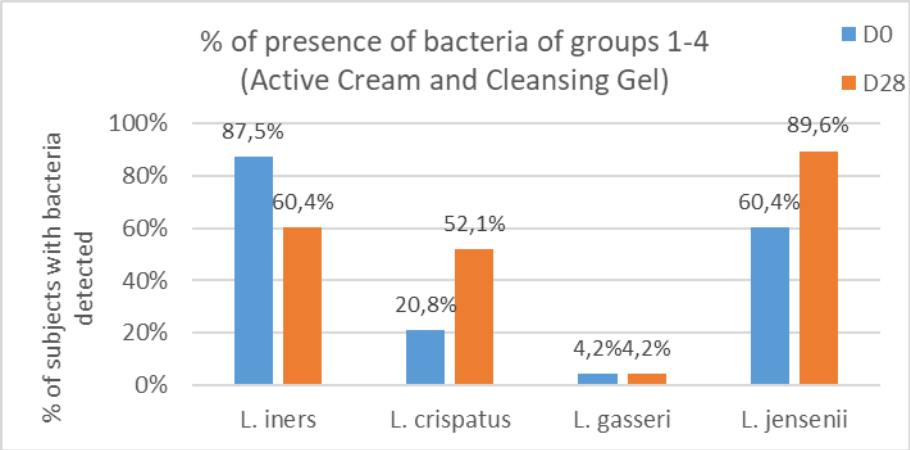


Bombax costatum extract was well perceived according to the questionnaire in terms of tolerance, reduction of irritation and itching sensations, reduction of dryness and improvement of comfort.

Anti-inflammatory properties of *Bombax costatum* extract observed by clinical scoring of erythema was confirmed by the significant decrease of inflammation biomarker IL1-α (-9.5%, $p<0.05$).

Total flora quantity was not modified by *Bombax costatum* extract. We observed a greater presence of *Lactobacillus crispatus* and *Lactobacillus jensenii* at D28 compared to D28 traducing a prebiotic effect. *Lactobacillus iners* presence is lower at D28 compared to D0, certainly due to the global restoration of microflora related to the increase of 2 previous bacteria. *Lactobacillus gasseri* has been detected at very low level. Observations on the presence of these 4 Lactobacillus tend to show a prebiotic effect of *Bombax costatum* extract.

Figure 8: % of presence *of Lactobacillus iners, Lactobacillus crispatus, , Lactobacillus gasseri and Lactobacillus jensenii* at D0 and D28 for groups 1 to 4 pooled.



Discussion & Conclusion.

We have demonstrated that a natural and plant polysaccharide-rich extract obtained from *Bombax costatum* is able to limit growth, adhesion and biofilm forming properties of a pathogenic strain (*S. aureus*), without affecting commensal bacteria. Moreover, it stimulates immune defenses and helps to preserve epidermal barrier from alteration due to *S. aureus*. These results show the beneficial properties of the extract to preserve skin microbiota homeostasis by promoting commensal against pathogenic bacterial strains and by preserving from the deleterious effects of *S. aureus*. The extract also diminishes adhesion of *C. xerosis* on explant and reduces the odors *in vitro* (data not shown). Finally, the extract promotes the growth of lactobacilli showing a prebiotic and protective effect of vaginal microflora.

In vivo clinical study also demonstrated the capacity of *Bombax costatum* extract to maintain the intimate area of women in good condition and to rebalance the intimate microflora.

Conflict of Interest Statement.

All authors are employees of Laboratoires Expanscience.

References.

1. Ceccarani et al. Diversity of vaginal microbiome and metabolome during genital infections. Scientifi Reports. 2019, 9:14095