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

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· 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 (BCP) was evaluated *in vitro* on different microbiota models showing its ability to preserve skin microbiota equilibrium. A clinical study has been performed showing its efficacy.

· Materials & Methods ·

In vitro evaluation

Bacterial strains:
Staphylococcus aureus; S. epidermidis; S. hominis; Lactobacillus gasseri, L. acidophilus, L. rhamnosus, L. crispatus and L. jensenii.

Bacterial growth:
A mixture of 3 bacterial strains were cultivated for 48h in presence of BCP. The growth of each bacterium was evaluated after subculture on specific agar.

Biofilm formation:
An aliquot of bacterial culture was deposited and incubated in presence of BCP for 24h. At the end of incubation, the biofilm was stained using crystal violet and quantified by optical density measurement.

Epidermal response study:
Reconstructed human epidermis (RHE), topically pre-treated for 24h by BCP, were incubated in presence of *S. aureus* secretum for 24h. Barrier markers immunostaining were performed.

Lactobacilli prebiotic effect studies:
Lactobacilli strains were cultivated for 4 or 24h in presence of BCP. The growth of each bacterium was evaluated after subculture on specific agar. The acid lactic production was measured in culture medium after 24h.
Reconstructed human vaginal epithelia (HVE) were colonized by *L. crispatus* and *L. gasseri* and, in parallel, treated by BCP for 6h. Then, HVE surface were observed by Scanning Electron Microscopy (SEM).

In vivo evaluation

A clinical study under gynecological control has been performed to evaluate the capacity of BCP to rebalance the intimate microflora which could be affected after vaginosis or mycosis pathologies, in case of irritations or in menopaused women.
5 groups of 10 subjects (group 1: post treatment for bacterial vaginosis, group 2: post treatment for mycosis, group 3: presenting vulvar redness / irritation and vaginal discomfort sensations without clinically proven pathology, groups 4 and 5: post menopause and complaining of a feeling of vulvar and vaginal dryness) used an active cream combined with active cleansing gel containing both 1.25% of BCP except group 5 which used control products.
Subjects used cream twice a day and cleansing gel once a day as a liquid soap on the intimate 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 cytokine) and micro-flora (total flora, *Lactobacillus crispatus*, *L. iners*, *L. jensenii* and *L. 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 effect on cutaneous microbiota

Regulation of bacterial growth and biofilm formation

BCP inhibited the growth of the pathogenic strain *S. aureus*, without significantly affecting the growth of commensal bacteria (Fig.1). The extract significantly inhibited *S. aureus* biofilm formation when it increased that of *S. epidermidis* (Fig. 2).

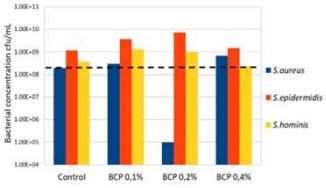

Figure 1: Bacterial growth after 48h
(Ratio [S.aureus]=1 / 100 [S.hominis]=[S.epidermidis])

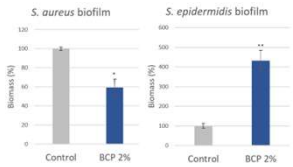

Figure 2: Evaluation of biofilm formation
** p<0,05; ** p<0,01; Test de Mann-Whitney*

Protection against *S. aureus*-induced damages to epidermal barrier

BCP, 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 (Fig. 3).

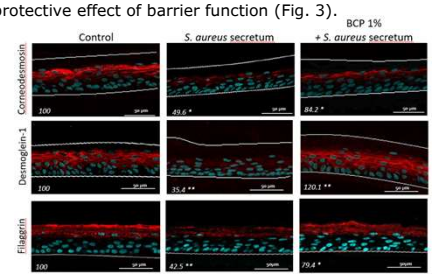

Figure 3: Fluorescent immunostaining of barrier markers in RHE
**p<0.05; **p<0.01 – Unpaired t test*

In vitro and in vivo effect on vaginal microbiota

In vitro, BCP induced the growth of good lactobacilli and inhibited the growth of two pathogen strains *(G. vaginalis - E. coli)* (Table 1). BCP, also, increased the lactic acid production by *L. acidophilus* (Fig. 4). Lactic acid is important to maintain an acid pH in this area, essential for maintaining a healthy vaginal microflora.

	L. crispatus (Log10 CFU/ml)		*L. jensenii* (Log10 CFU/ml)		*G. vaginalis* (Log10 CFU/ml)		*E. coli* (Log10 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 1: Evaluation of viability of vaginal bacteria * p<0,05 ; ** p<0,01 vs Control One way ANOVA followed by Tukey test.*

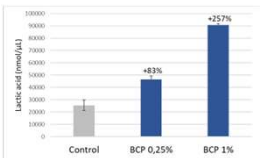

Figure 4: Lactic acid production by L. acidophilus.

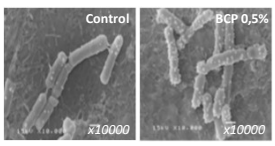

Figure 5: Images of HVE surface (SEM observation)

BCP induced the formation of membrane microvesicles (MV) on lactobacilli (Fig. 5). These MV are associated with the probiotic properties of these bacteria by activating the immune system and host defenses and promoting regulation of the bacteria community [2]. These results suggest that BCP could promote communication with the host in favor of prebiotic and protective effect.

In vivo, BCP significantly improved erythema, dryness, fissure and global irritation assessed by clinical scoring and burning sensation, pruritus and dyspareunia expressed by the subjects themselves (Fig. 6). pH was not disturbed and stayed in normal physiological range. Limit statistically difference (p<0.1) in favor of BCP was observed between group 4 and 5 for the dryness.

BCP 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 BCP 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 BCP. We observed a greater presence of *L. crispatus* and *L. jensenii* at D28 compared to D28 traducing a prebiotic effect (Fig. 7). *L. iners* presence is lower at D28 compared to D0. *L. gasseri* has been detected at very low level.

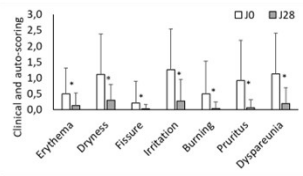

*Figure 6: Clinical and auto-scoring at J0 and J28 for groups 1 to 4 pooled. * p<0,05.*

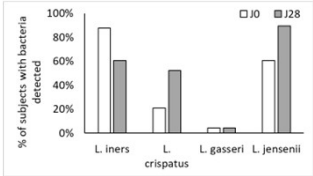

Figure 7: % of subjects with bacteria detected at J0 and J28 for groups 1 to 4 pooled.

· 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 without affecting commensal bacteria. Moreover, it stimulates immune defenses (data not shown). These results show the beneficial properties of the extract to preserve skin microbiota homeostasis. The extract also promotes the growth of lactobacilli showing a prebiotic and protective effect of vaginal microflora. *In vivo* clinical study also demonstrated the capacity of extract to maintain the intimate area of women in good condition and to rebalance the intimate microflora.

· References ·

1. Ceccarani *et al.* Diversity of vaginal microbiome and metabolome during genital infections. Scientific Reports. 2019, 9:14095
2. Dean *et al.* Isolation and characterization of Lactobacillus-derived membrane vesicles. Scientific Reports. 2019, 9:877