

# Gynandropsis gynandra extract limits development of acne by preserving microbial balance and associated virulence factors

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## Introduction

Acne in adult women has increased in recent years and remains a major concern. Often associated with an overgrowth of the bacteria *Cutibacterium acnes* (*C. acnes*), acne would be mainly due to a loss of diversity and an imbalance between healthy and acne-prone strains of this bacteria (dysbiosis)<sup>1</sup>. *C. acnes* strains are classified into six main phylotypes (IA1, IA2, IB, IC, II and III) (Fig.1)<sup>2</sup>. Some phylotypes such as IA are related to acne and others phylotypes such as II are related to healthy skin.



Figure 1: Summary of the nomenclatures of *C. acnes* phylotypes and their association with acne and healthy skin<sup>2</sup>.

Clade (Based on Whole-Genome Sequencing)	Clade (Based on Belfast eMLST (38))	Clade (Based on Ashrus MLST (39))	RT [30]	Acne	Healthy Skin
IA-1	IA-1	I-1a	RT1	✓	✓
IA-2	IA-1	I-1a	RT4, RT5	✓	✓
IB-1	IA-1	I-1b	RT8	✓	✓
IB-2	IA-2	I-1a	RT3	✓	✓
IB-3	IB	I-2	RT1	✓	✓
IC	IC	NA	RT5	✓	✓
II	II	II	RT2, RT6	✓	✓
III	III	III	NA	✓	✓

eMLST: expanded multi-locus sequence typing; MLST: multi-locus sequence typing; NA: not assigned; RT ribotype.

In addition, *C. acnes* produces virulence factors (biofilm, porphyrins) which contribute to the pathogenic character of these strains and are involved in the virulence of acne. Porphyrins are metabolites that can generate oxidative stress and induce inflammation in keratinocytes<sup>1</sup>. We have obtained new results which confirm the efficacy of our active ingredient from *Gynandropsis gynandra* (GG) to restore skin microbiota balance and fight acne.

## Materials & Methods

### Bacterial growth study

A mixture of commensal and pathogenic bacterial strains, representative of cutaneous microbiota, was cultivated in a liquid medium for 48 hours with GG extract or the positive reference Phenonip. The growth of each bacterium strain was evaluated after sub-culture on specific agar.

### Biofilm study

Biofilm formation of two strains of *C. acnes* (phylotypes I and II) was studied using crystal violet dye.

### Porphyrins quantification

Two strains of *C. acnes* (phylotypes I and II) were incubated with GG extract for 48 hours. Porphyrins assay was performed: bacterial cultures were extracted with a mixture of ethyl acetate/acetic acid. After centrifugation, the aqueous phase was recovered to measure the absorbance at 405 nm.

### Squalene oxidation (in tubo test)

Squalene was incubated with ozone and active ingredient. Squalene and its metabolites (C27-pentaenal, C22-tetraenal, C17-trienal) were extracted and quantified by gas chromatography-mass spectrometry.

## Results

### Regulation of bacterial growth

GG extract inhibited the growth of pathogenic strain of *C. acnes* with a bactericidal effect at 1%. GG extract had no effect or even a positive effect on the commensal strains *S. epidermidis* and *S. hominis*. GG extract tended to inhibit the growth of the pathogenic strain *S. aureus* at 1% (Fig.2).

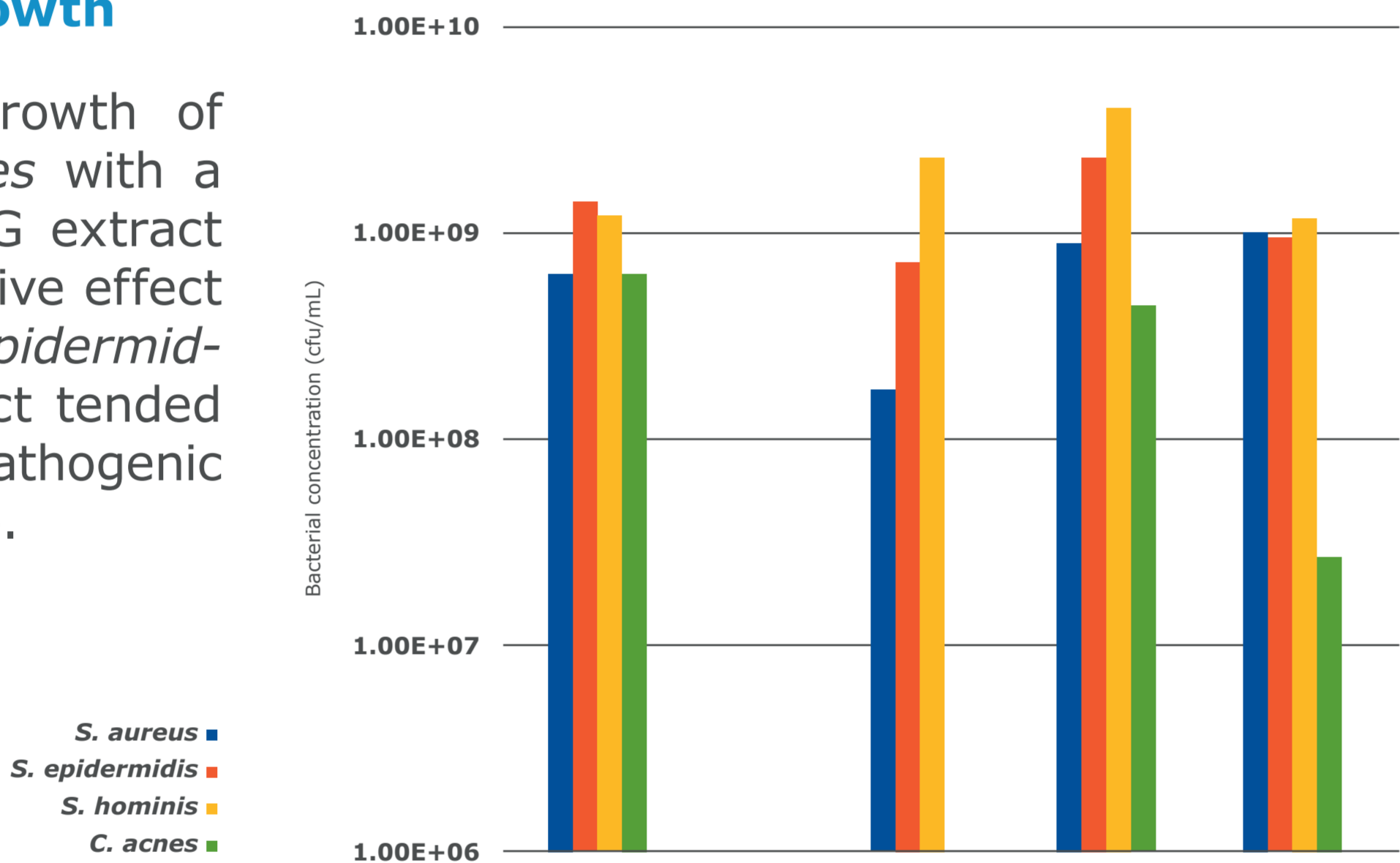


Figure 2: Evaluation of bacterial growth

### Inhibition of biofilm formation

GG extract tended to inhibit the formation of the biofilm of the acne-associated strain of *C. acnes* without effect on the non-acneic strain (Fig.3).

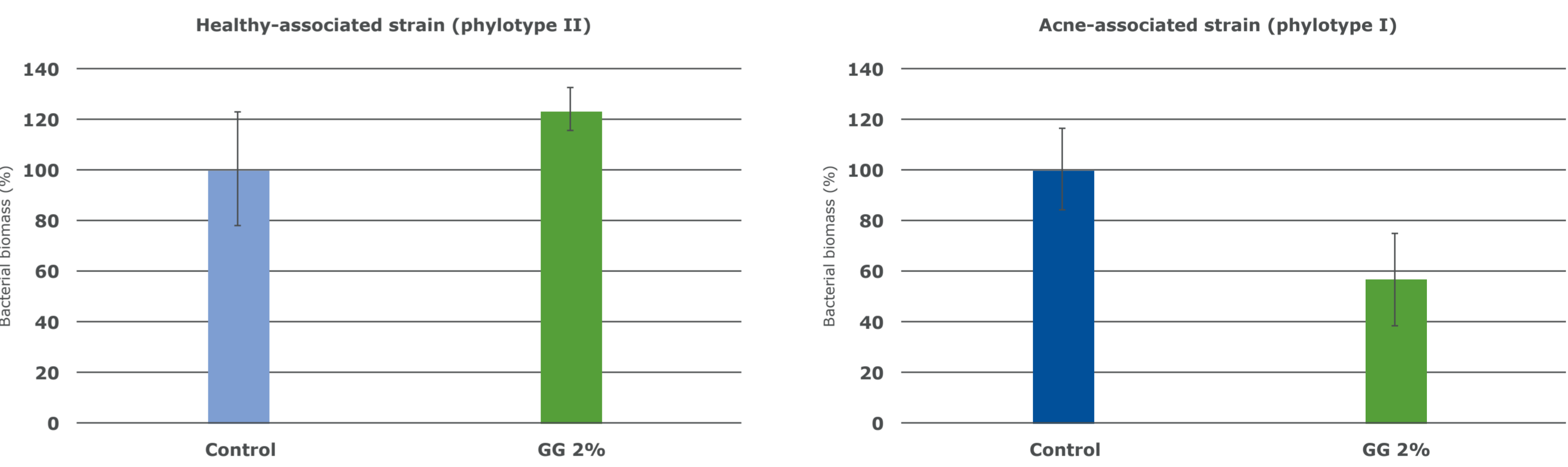


Figure 3: Evaluation of biofilm formation

### Inhibition of porphyrins production

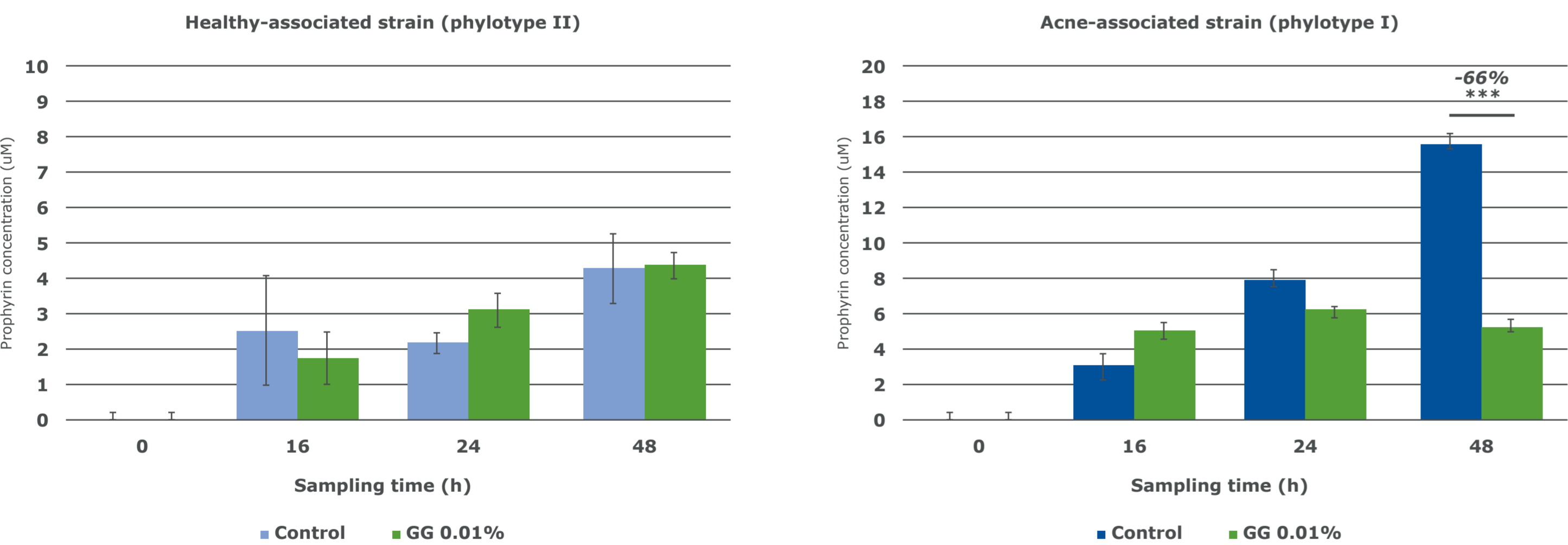


Figure 4: Evaluation of porphyrins production  
Student t test, \*\*\* p<0.001

### Inhibition of squalene oxidation

	Squalene (µg/ml) (CV%)	Protection (%)	Squalene Metabolites (µg/ml) (CV%)	Protection (%)
Control	76,3 (0,3)	/	Not detected	/
Ozone	31,5\$\$\$ (4,2)	/	18,0 (6,6)	/
Vitamine E 0.1% (Squalene + ozone + Vit E)	43,1*** (5,3)	36,9	9,8*** (5,8)	45,4
GG 0.075% (Squalene + ozone + GG)	37,1** (2,8)	17,8	31,3*** (4,5)	25,8
GG 0.15% (Squalene + ozone + GG)	42,1*** (2,9)	33,9	11,5*** (5,7)	36,1

Table 1: Quantification of squalene and its metabolites  
One way ANOVA followed by Tukey test  
\*\*p<0,01 ; \*\*\*p<0,001 vs ozone; \$\$\$p<0,001 vs control

GG extract strongly inhibited the production of porphyrins at 48 hours by acne-associated strain of *C. acnes* and did not modulate the concentration of porphyrins produced by the healthy strain (Fig.4).

These results show a specific effect of GG extract on the acne-associated strain.

GG extract showed a protective effect against the degradation of squalene. It limited ozone-induced squalene degradation as well as the generation of primary metabolites related to squalene oxidation (Tab.1).

Oxidative stress can lead to inflammatory and pro-comedogenic mechanisms. GG extract can help to modulate this oxidative stress observed with acne.

## Conclusion

These new *in vitro* results demonstrate the capacity of GG extract to limit the development of acne by a specific action on acne-associated strain of *C. acnes*.

## References

<sup>1</sup> Dréno B, et al. *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates. Journal of the European Academy of Dermatology and Venereology. 2018;32(2),5-14.  
<sup>2</sup> Lee, Y.B; Byun, E.J and Kim, H.S. Potential Role of the Microbiome in Acne: A Comprehensive Review. Journal of Clinical Medicine. 2019,8,987.

