Modulation of hyperseborrhea by targeting inflammatory pathways involved in acne pathogenesis: *in vitro* and *in vivo* studies

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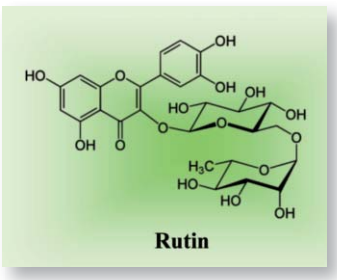
Introduction

Acne is a chronic disorder of the pilosebaceous unit in which inflammation plays a central role. Indeed, inflammation represents an early event, and may be further amplified by both sebum lipid alteration and *Propionibacterium acnes* (*P. acnes*) proliferation.
Sebum is not only produced in excess in acne (hyper-seborrhea), but its composition is also altered with an accumulation of oxidized squalene and a deficit in linoleic acid, creating an inflammatory ground.
P. acnes contributes to exacerbate inflammation by activating the membrane receptor TLR2 (Toll-Like Receptor 2) on keratinocytes and consequently stimulating the production of inflammatory mediators, such as Interleukin-8 (IL8), chemoattractant for immune cells (neutrophil, lymphocyte).



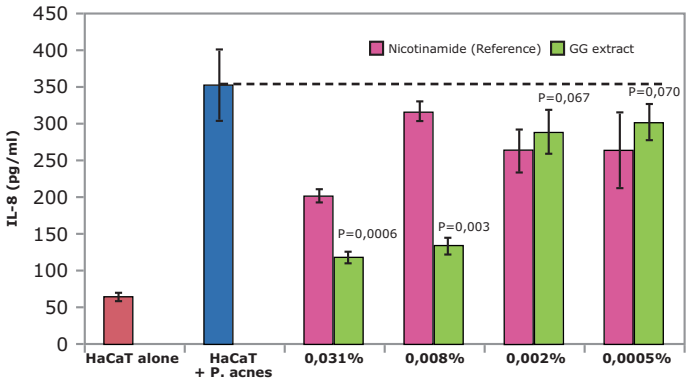
We have developed a plant extract from *Gynandropsis gynandra* (GG) or *Cleome gynandra*, enriched in polyphenols (mainly rutin and hydroxycinnamic acid), according to an optimized, secured and eco-designed process, in compliance with our sustainable policy. We have demonstrated *in vitro* and *in vivo* the capacity of this active ingredient to target different pathways involved in acne pathogenesis.

In vitro biological activity

The GG extract can help the skin to fight against *P. acnes* colonization and its consequences: we have shown a strong and direct inhibitory effect on *P. acnes* growth (data not shown) and a significant inhibition of IL8 production induced by *P. acnes* in keratinocytes.
This anti-inflammatory action was further supported by the capacity to significantly inhibit neutrophils migration and LTB4 release (not shown) as well as a high inhibitory effect on TLR2 expression and IL8 release induced in skin organ culture by arachidonic acid.

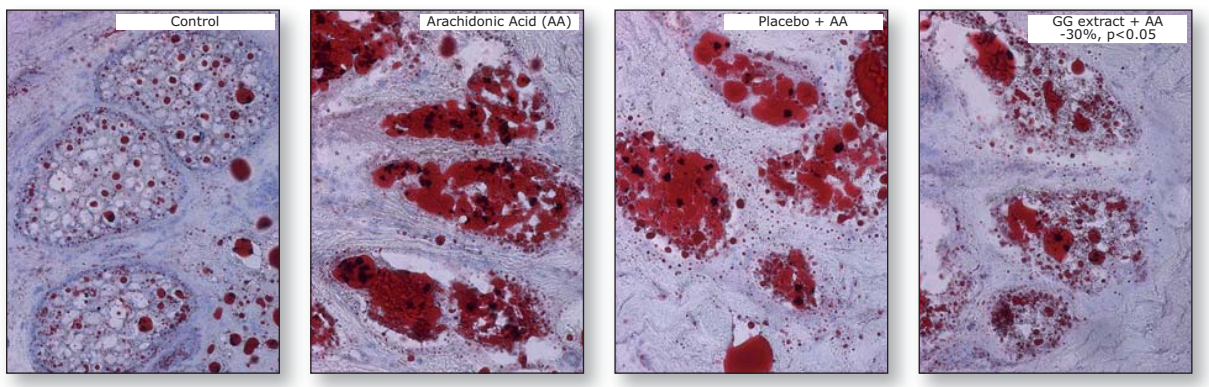
Besides, GG extract has an important anti-seborrhea potential: in skin organ culture it decreased sebaceous glands lipid content. Furthermore, with its capacity to inhibit lipid peroxidation, it could limit oxidative alteration of sebum composition occurring in acne.

Inhibition of IL8 release by keratinocytes in response to *P. acnes*

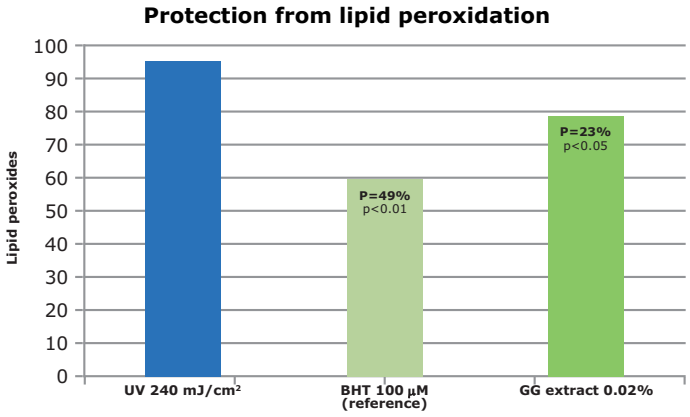


Human HaCaT keratinocytes, pre-incubated in presence of the GG extract, were co-cultivated with P. acnes, the release of interleukin-8 was evaluated by ELISA. Student t test.

Modulation of lipid production in sebaceous gland



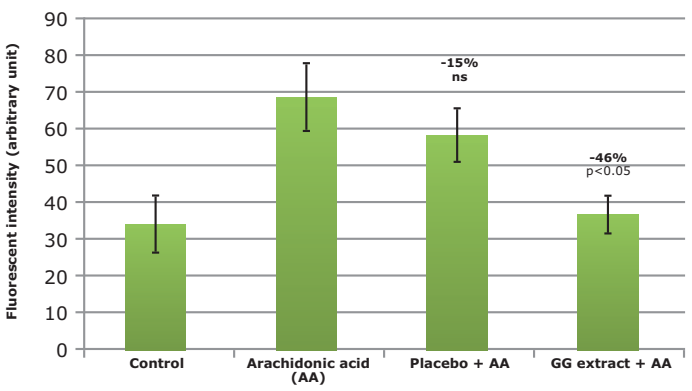
Protection from lipid peroxidation



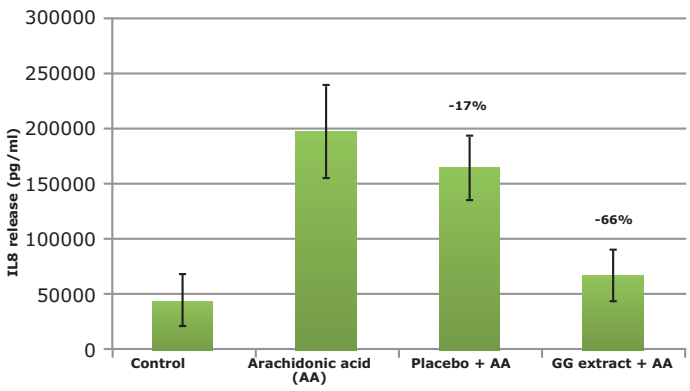
Jurkat cells, pre-treated by the GG extract, were submitted to UV irradiation, lipid peroxidation was then evaluated by flow cytometry thanks to the fluorescent probe C11-fluor. P=Protection; Student t test.

Anti-inflammatory activity

TLR2



IL8

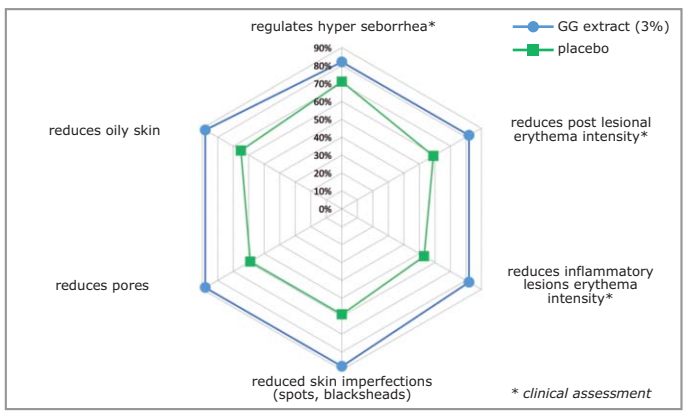


GG extract formulated at 3% in a cosmetic cream or its placebo were topically applied on human full-thickness scalp skin organ cultures (hSOC) which were stimulated by arachidonic acid.
Lipid production within the sebaceous gland was evaluated by oil red O staining on cryosection; inflammatory response was evaluated by TLR2 immunostaining and IL8 ELISA assay.

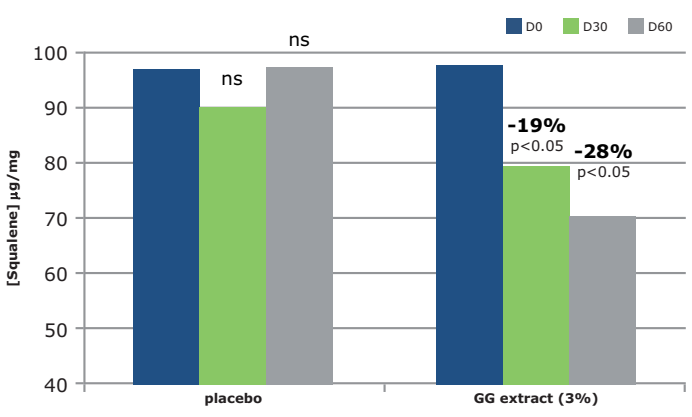
Confirmation of *in vivo* efficiency

Protocol
We conducted a double-blind randomized placebo-controlled study on 34 women (2 x 17 subjects) with mild to moderate acne (retentional lesions <12; inflammatory lesions: 5-7) during 60 days. The GG extract was introduced at 3% in the active formula. A non-invasive sampling method was used to collect the skin surface lipids on forehead. Squalene and oxidized squalene were quantified respectively by GC/MS and LC/MS at 0, 30 and 60 days of application of the products. Results are expressed in µg per mg of total lipids collected on the area of interest. A global clinical assessment and self-evaluations were performed at 60 days.

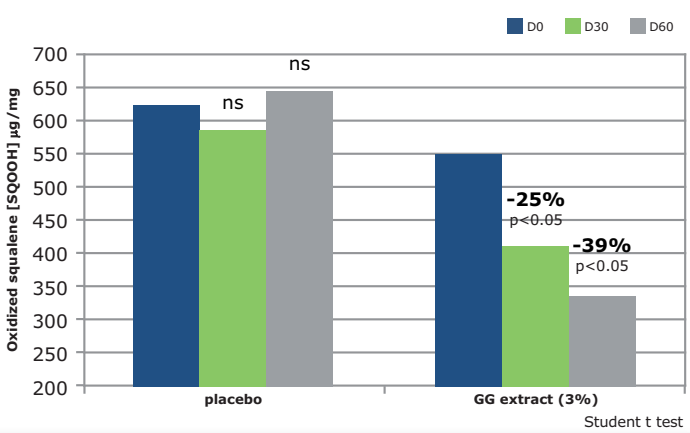
Global clinical assessment and self evaluation after 60 days



Seboregulation activity
Squalene



Anti-oxidant activity
Oxidized squalene



Student t test

We observed highly significant differences between the 3% GG extract formula and placebo on squalene and oxidized squalene at 30 and 60 days.
Global assessment by the clinician and self-evaluation by subjects also showed significant differences in favor of the formula containing the plant extract.

Conclusion

By these *in vitro* and *in vivo* investigations, we underlined the capacity of the *Gynandrospis gynandra* extract to modulate the main inflammatory-related pathways of acne pathogenesis, regulate sebum secretion, improve sebum quality by protecting bounded fatty acids and limit oxidative alterations of the skin. Thus, this newly developed plant extract offers a global solution for the management of oily and acne prone skin.