In vivo evaluation of skin volatile organic compounds and squalene peroxidation on stressed and aged skin

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Abstract

Background Volatile organic compounds (VOCs) are emitted from various natural and chemical sources. Some VOCs may have therapeutic benefits, but others may have harmful effect. The human body also emits VOCs (via breath, skin…) which represent the footprint of the cellular activity and can thus reveal certain dysregulation of the metabolism. These emanations depend on the physiological status as they may vary. Skin VOCs arise from eccrine, sebaceous and apocrine gland secretions and their interactions with microbiota determine our body odors. Specific skin VOCs such as the aldehydes 2-nonenal (Asian skin) and nonanal (Caucasian skin) increase with age. These VOCs derive from lipid peroxidation which increases with age and oxidative stress.

Methods: A clinical study was designed to evaluate the variations of nonanal, on subjects applying on the face a formulation containing a botanical extract vs placebo. The study enrolled 25 stressed Caucasian volunteers, aged from 36 to 66 yo.

Results: After one month of application twice a day, the use of the extract-formulation was associated with a decrease of squalene peroxidation and nonanal emanations compared to placebo. In parallel, the skin luminosity (known to be influenced by lipid peroxidation) and fine lines parameters were evaluated. An improvement of skin luminosity as well as an attenuation of fine lines parameters were noticed on the extract-formula-treated side, compared to placebo.

Conclusion: This study pointed out the concomitant decrease of both skin lipid peroxidation level and nonanal emanation level, associated with the application of the formula in aged or stressed population**.**

Keywords: VOC, nonanal, skin aging, lipid peroxidation

Introduction.

Volatile organic compounds (VOCs) are emitted from various natural and chemical sources. Some VOCs may have therapeutic benefits (e.g., the VOCs emitted by forests, usually odorant and pleasant) [1]. Others may have harmful effect, like the ones due to solvents, adhesives, fuels, or industrial wastes[2]. The human body also emits various VOCs (via breath, skin…) which represent the footprint of the cellular activity and can thus reveal certain dysregulations of the metabolism. These emanations depend on the physiological status as they may vary with the donor, the gender, with aging, the diet and the microbiota[3]. Skin VOCs arise from eccrine, sebaceous and apocrine gland secretions and their interactions with microbiota determine our body odors. Specific skin VOCs such as the aldehydes 2-nonenal (Asian skin) and nonanal (Caucasian skin) increase with age. These VOCs derive from lipid peroxidation which increases with age and oxidative stress[4-5].

The present study was conducted to evaluate the relationship between lipid peroxidation and VOCs *in vivo*. For this, a botanical extract from *Santalum album* (*S. album*) known for its effects on olfactory receptor OR2AT4 and advanced glycation ends products (AGEs), *in vitro* was selected to evaluate in the same time its effect on lipid peroxidation and VOCs as well as its anti-aging impact.

Materials and Methods.

A clinical test has been designed to study the variation of nonanal as well as other skin parameters degraded with age and oxidative stress. 25 stressed Caucasian volunteers (selected thank to a questionnaire), aged from 36 to 66 years-old, were enrolled. They applied on the face, for 28 days, twice a day, a cream containing 1% *Santalum album* (*S. album*) extract on one side and placebo on the other side. The effect on oxidative stress has been first assessed by measuring nonanal and lipid peroxidation. The nonanal was sampled on the face by rolling a SBSE (Stir Bar Sorptive Extraction) on the cheek and analyzed by GS/MS. The lipids have been collected with sebutape applied on the forehead and quantified by HPLC/MS. Secondly, skin luminosity, known to be influenced by lipid peroxidation was evaluated by spectrophotometry near the nose. To evaluate the effect on age, fine lines' parameters and skin roughness have been carried out on the crow's feet area by the combination of stereometry and fringe projection technology (AEVA® - Eotech®).

Moreover, volunteers were asked to evaluate the state of their skin according to a scale from 0 to 100, where 0 was the worst and 100 the best state, excepted for the evaluation of the

crow's feet fine lines where it was the contrary. All the measurements were done the first day of the study, before cream applications (D0) and at the end of the study, after 1 month of applications (D28). The results were analyzed using the Student's *t*-test or Wilcoxon test depending on whether the data followed a normal distribution or not at determined times.

Results.

After one month of application twice a day, the use of the formulation was associated with a decrease of squalene peroxidation and nonanal emanation compared to placebo (figure 1).

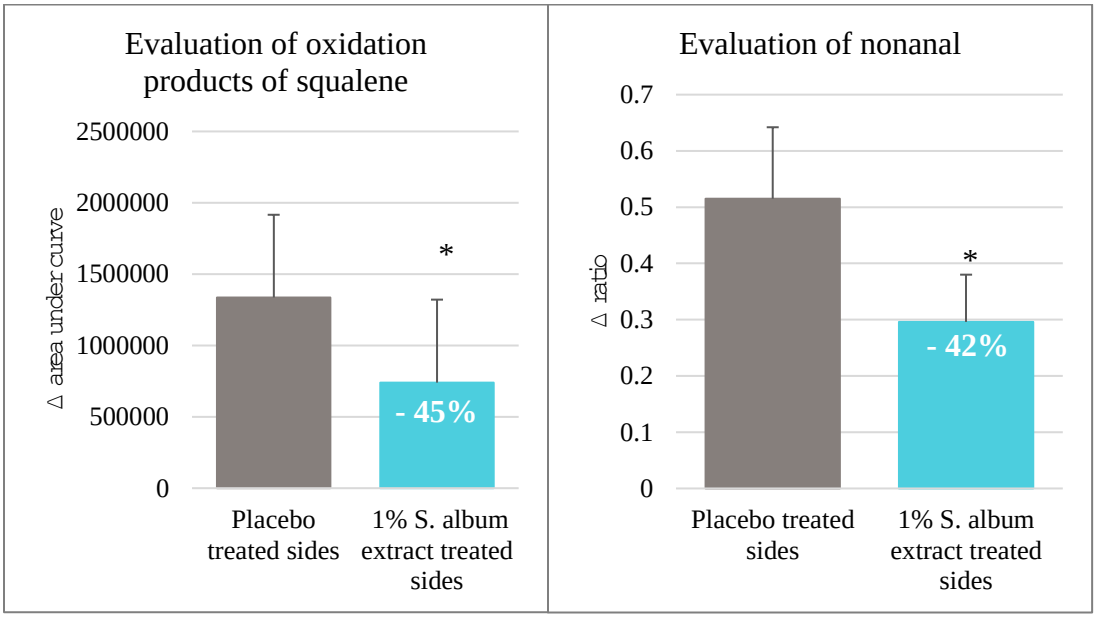

Figure 1: **Measurements of oxidation products of squalene and of nonanal, difference between D28 and D0, for the 1% *S. album* extract treated sides and placebo treated sides**

*: significant with Student's t-test or Wilcoxon test depending on whether the data followed a normal distribution or not. n=23 +/- sem for oxidation product and n=22 +/- sem for nonanal

The concomitant decreases of oxidation product of squalene and nonanal highlighted that, both may be correlated and by decreasing skin lipid peroxidation with *S. album* extract, we could minimize the nonanal quantity at the skin surface.

In addition to the decrease of lipid peroxidation, the anti-oxidant effect could be highlighted by an enhancement in skin luminosity. After one month of application with the cream containing 1% *S. album* extract compared to placebo, an improvement of 46% of skin luminosity was noticed. (Figure 2).

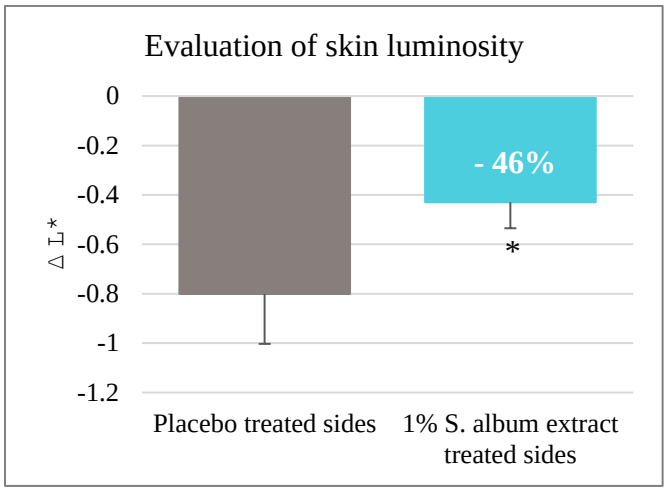

Figure 2: **Measurements of skin luminosity, difference between D28 and D0, for the 1% *S. album* extract treated sides and placebo treated sides**

*: significant with Student's *t*-test or Wilcoxon test depending on whether the data followed a normal distribution or not; n=25 +/- sem

The improvement in skin luminosity supported the antioxidant effect observed in the previous result and by the way the decrease in nonanal influenced by skin aging.

Concerning the anti-aging effect, different skin topography parameters were evaluated. First, fine lines' parameters were assessed, and a statistical decrease of these latter were noticed after 1% *S. album* extract-containing cream applications compared to placebo. This effect was visible on color pictures showing less visible fine lines and wrinkles on side treated with the formula containing 1% *S. album* extract compared to placebo treated side (Figure 3 & 4).

Evaluation of fine lines' parameters

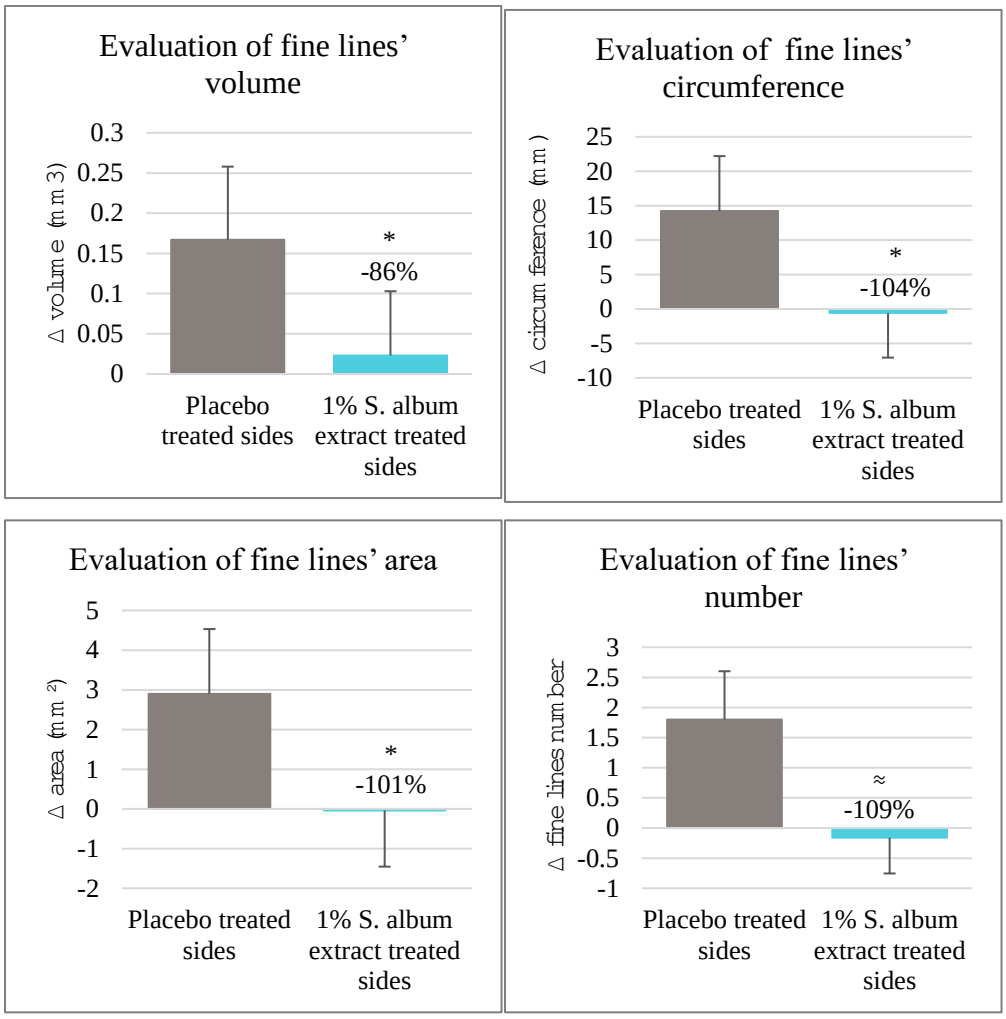

Figure 3: **Measurements of fine lines' parameters, difference between D28 and D0, for the 1% *S. album* extract treated sides and placebo treated sides.**
≈: directional; *: significant; with Student's *t*-test or Wilcoxon test depending on whether the data followed a normal distribution or not, n=25 +/- sem

Color pictures of crow's feet area

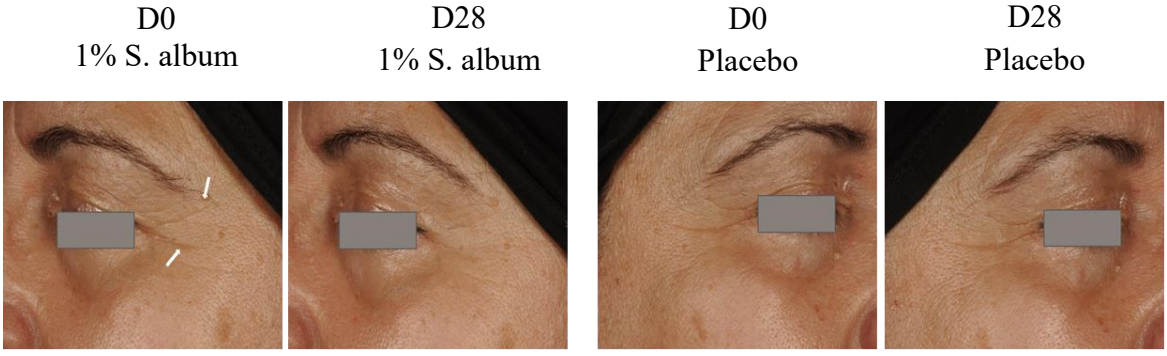

Figure 4: **Color pictures of one of the best volunteers (#28; woman; 60 year-old)**

The reduction of fine lines may highlight the smoothing and relaxing effect of *S. album* extract on skin features.

Secondly, the skin roughness was carried out by the evaluation of Ra (arithmetic average of the absolute values of the roughness profile ordinates) and Rq (root mean square average of the roughness profile ordinates). After 1 month of application with 1% *S. album* extract containing cream a significant decrease of roughness parameters was observed compared to the placebo side. This result could be appreciated on the 3D pictures, where the microrelief was less pronounced on 1% *S. album* extract treated side and also on color pictures (Figure 5 & 6).

Measurement and 3D representation of skin smoothness

Parameters	treated sides	Mean (D28-D0)	p	% of change
Ra (mm)	Placebo	0.00096	0.0059**	-92%
	1% *S. album* extract	0.00008		
Rq (mm)	Placebo	0.00108	0.0292*	-93%
	1% *S. album* extract	0.00008		

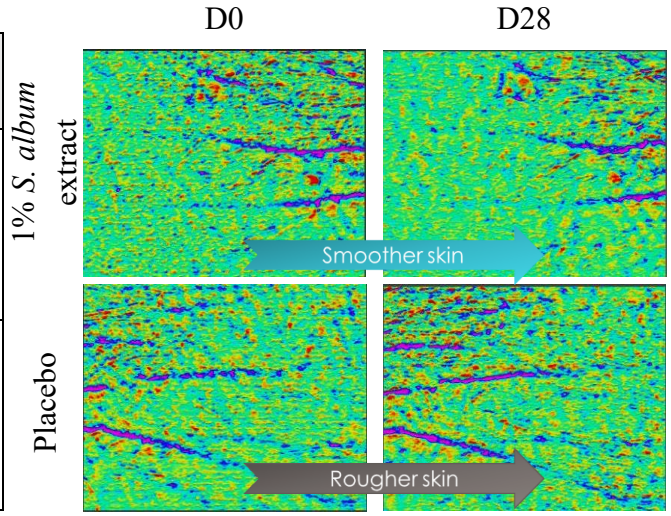

Figure 5: **Measurements and 3D representation of skin roughness (# 17; man; 61 year-old).**
For measurements, difference between D28 and D0, for the 1% *S. album* extract treated sides and placebo treated sides.
*: significant and **: very significant with Student's *t*-test or Wilcoxon test depending on whether the data followed a normal distribution or not, n=25 +/- sem

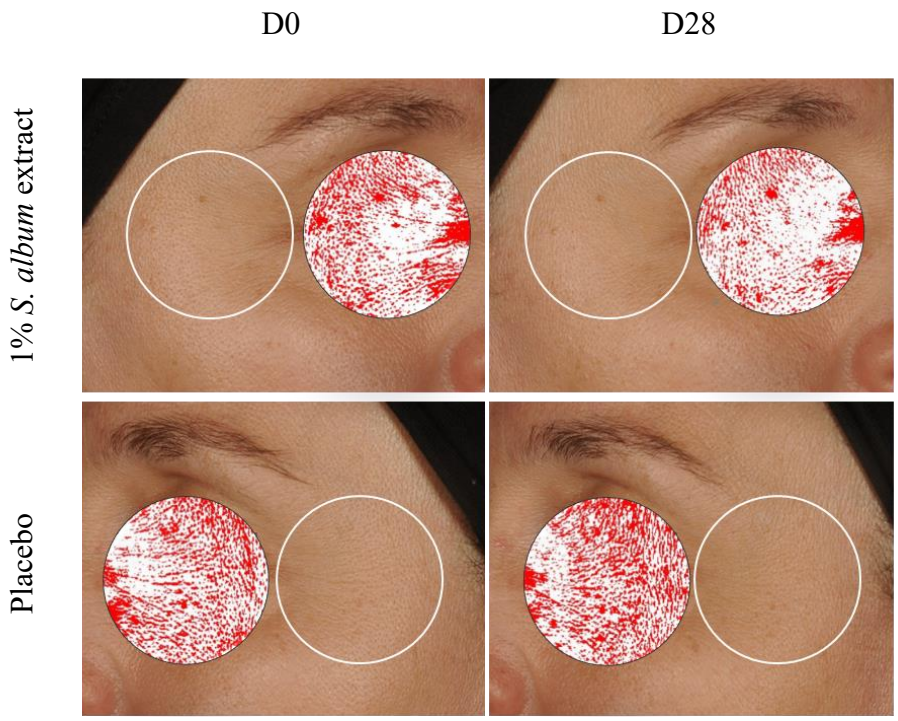

Figure 6: **Color pictures of skin smoothness (# 15; woman; 36 year-old)**

The comparison of the results between both sides (placebo *versus* treated) showed a positive impact on visible fine line's parameters such as the volume and circumference but also on skin smoothing demonstrated the anti-aging and relaxing effect of the extract *S. Album*.

To confirm all the results obtained with objective measurements, the volunteers performed a survey at each control visit. They rated higher scores for skin hydration, firmness and less fine lines on the side treated with 1% *S. album* extract, compared to the placebo side (Figure 7).

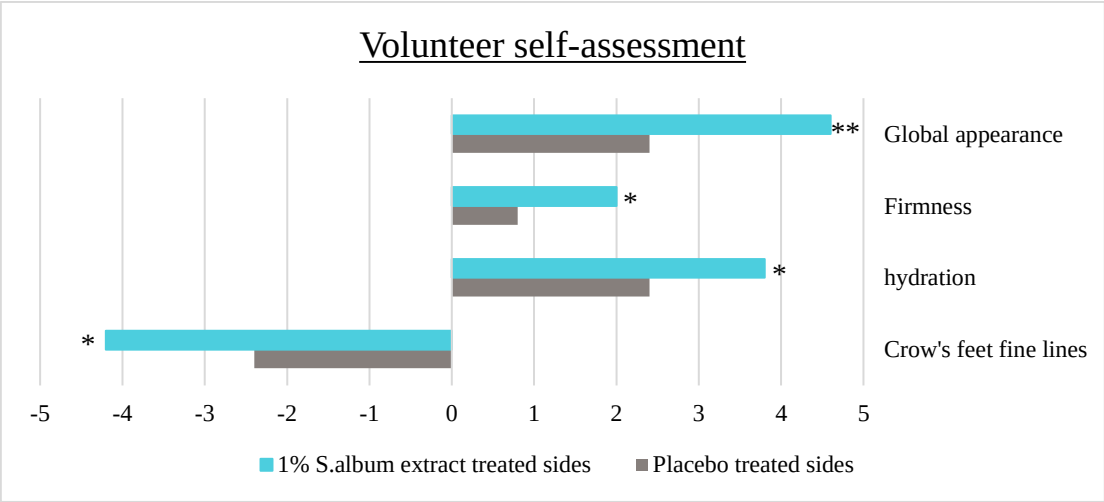

Figure 7: **Volunteer self-assessment, difference between D28 and D0, for the 1% *S. album* extract treated sides and placebo treated sides**

*: significant; **: very significant with Student's *t*-test or Wilcoxon test depending on whether the data followed a normal distribution or not; n=25

This last result supported all the previous data, showing an ability on *S. album* extract to provide skin with a visible and perceivable smoother and healthier look.

Conclusion. This study pointed out the concomitant decrease of both skin lipid peroxidation level and nonanal emanation level, associated with the application of the formula in stressed population. These diminutions have been gone with an improvement in skin luminosity and skin smoothness, highlighted the potential effect of the extract to counteract the effect of aging and oxidative stress.

Conflict of Interest Statement.

NONE

References

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