A cosmetic ingredient able to repair the skin through skin melatonin pathway and sleep quality improvement

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Abstract

Introduction: Sleep is one of the most important elements to health. Overnight, skin goes into repair mode after daily stress, where melatonin plays a key role. Toxins are removed, cellular repair is increased as well as growth hormones production to improve skin quality during that period. In the current study, we investigated the impact of an active ingredient, a natural essential oil, on both sleep quality and skin regeneration, through an ex-vivo study.

Methods: Participants' sleep was measured using a non-contact monitoring device for 5 consecutive nights after application with the active or a placebo face cream. Moreover, an *ex-vivo* study was conducted with this ingredient to observe its skin regenerative effects after a daily stress induction. 25 genes associated with skin repairing and melatonin pathway were evaluated, and their modulation were confirmed by immunostaining.

Results: The in-context sleep studies revealed that the active significantly improved the total sleep duration by nearly 10 minutes ($p<0.05$) as compared to the placebo, which can primarily be attributed to a significant increase in deep sleep duration (5 minutes, $p<0.05$). The *ex-vivo* study also revealed its capacity to regenerate the skin after daily stressed-induction with a decrease of inflammatory (IL6, COX-2) and DNA damage marker (OGG1) and an increase of antioxidant enzymes (CAT, GPX1) and collagen. Interestingly, this product also modulated melatonin-associated genes (ASMTL, SLC15A1 or RORα).

Conclusion: Theses results demonstrate that our selected essential oil favorize skin regeneration process using a dual approach that acts on both sleep and skin improvement.

Keywords: Sleep, essential oil, skin regeneration, well-being

Introduction.

Over recent years an increasing number of reports document difficulties with initiating or maintaining healthy sleep patterns. This trend has been exacerbated by the global COVID-19 pandemic and is now widely recognized as a global public health crisis. Sleep patterns are not only increasingly being disrupted by behavioral, cognitive and social stressors, but also by digital technology stressors such as blue light, emitted from the screens that have become a pervasive part of the modern lifestyle [1]. Disruption of sleep patterns due to lifestyle-related stressors can lead to negative acute and chronic health outcomes that create an enormous personal and economic strain on society. For example, outcomes such as daytime sleepiness, fatigue, mood disturbances, impaired cognitive performance and productivity [2], as well as an increased risk of cardiovascular disease [3], type-2 diabetes [4], hypertension [5] and mortality [6] not only have personal consequences but also place an enormous burden on families, healthcare systems and respective budgets. Therefore, developing consumer products with proven functional benefits to improve sleep quality are of utmost importance and a high priority across industry sectors.

The skin functions as a barrier against the external environment and thus plays a crucial role in maintaining autonomic homeostasis. A lack of sleep degrades the quality of one's skin, such that sleep deprived people are rated as less healthy and less attractive than fully rested individuals [7]. Notably, after sleep deprivation, individuals were perceived with darker circles under the eyes, paler skin and with more wrinkles. In addition, attributes such as rough, dull and dry skin are often observed. Importantly, sleep deprivation increases the production of glucocorticoids, which are thought to alter the integrity of the lamellar bodies and thus impair the integrity of the skin barrier [8,9]. Further studies show that a lack of sleep leads to increased secretion of stress hormones such as cortisol or the Substance P neuropeptide [10,11]. Both Cortisol and Substance P are known to play a substantial role in acne development [12,13]. Similarly, it has been observed that an increase of inflammatory cytokines and tumor necrosis factor-α observed after sleep deprivation impede recovery of skin barrier function [14]. Together, these effects are thought to induce skin dehydration, trans-epidermal water loss (TEWL) and a loss of skin elasticity and skin tone, thereby exacerbating the signs of intrinsic ageing [15].

Melatonin, the main hormone released by the pineal gland, is critical for regulating the sleep cycle by modulating the circadian clock. But melatonin is also synthesized in many tissues other than the pineal gland, including human skin. [16]. L-tryptophan is the melatonin precursor, and it is enzymatically converted to melatonin via a well-known pathway [17]. A broad range of biological functions has been identified for melatonin; for instance, antioxidant with a decrease of ROS production and an increase in the expression of antioxidant enzymes or anti-inflammation by acting through NF-κB or cyclooxygenase-2 [18]. Some of these processes are mediated through interactions with its specific G protein-coupled receptors (MT1 and MT2) and also possibly via nuclear and cytoplasmic partners that include retinoid-related orphan nuclear hormone receptor family. Other functions of melatonin are receptor independent [19]. Therefore, targeting melatonin pathway represents an interesting pathway to improve skin condition.

An increasing number of research studies demonstrate the efficacy of aromatherapy for improved sleep quality and cognitive function using specific scents. A recent study by Lee et al. [20] showed that a blend of lemon, eucalyptus, tea tree, and peppermint significantly improved sleep duration and overall sleep quality. Li-Wei et al. [21] also reported a positive effect of lavender to promote deep sleep and reduce nighttime awakenings. However, interpretations are limited by widely differing methodological approaches including dosage levels used as well as a lack of rigorous control conditions. Skincare products also commonly incorporate essential oils, not only for fragrant properties, but also for their reported health benefits and antimicrobial properties [22]. For example, EO applications include anti-acne, skin-lightening and sun protection [23-26]. Clearly more research is needed to evaluate the efficacy of the putative benefits of essential oils in skincare products.

In the current study, we introduced a novel dual approach to improve regenerative skin by acting on both sleep and skin. The efficacy of a single EO was evaluated on sleep quality, administered at low dosage levels without perceptible smells in a face cream application, relative to a non-scented control condition. Sleep quality was measured in healthy consumers using a biometric measurement platform validated against the gold standard of polysomnography (PSG) and designed for use in a real-world setting. In addition, skin *ex-vivo* experiment was conducted to further explore the repairing activity on skin of the same oil after daily stress. We also observed the capacity of the active to act on the melatonin

pathway. Results from both *in-vivo* and *ex-vitro* studies demonstrated a dual effect of this specific EO significantly improving sleep quality and revealing a repairing effect on skin cells against stressors.

Materials and Methods.

***In-vivo* study – Measurement of sleep quality**

Participants:

32 healthy participants (16 males, 22-56 years, Median age 41.5 years) with self-reported mild sleep disturbances caused by lifestyle factors, but without a clinical diagnosis of a sleep disorder, were recruited for the study. Participants self-reported a normal sense of smell and no known allergies. All study procedures including participant recruitment was reviewed and approved by the Western Institutional Review Board (WIRB).

Materials:

All materials were reviewed and approved by the Global Regulatory Affairs (GRA) department at IFF for safe use with human subjects in face cream application at the specified dosages. Each study consisted of 2 separated face cream samples, one active and one control, to be used across 3 separate weeks. 1% of active ingredient (EO diluted in Triheptanoin) was mixed in a generic unscented face cream product base. For the control condition, solvent was mixed at 1% in a generic unscented face cream.

Devices:

For objective sleep measurements, participants used the SleepScore Max device (SleepScore Labs, Carlsbad, CA), a non-contact monitoring device that uses radio frequencies to track and stage sleep. Importantly, the SSL technology platform has been validated against Polysomnography (PSG), the gold standard of sleep measurement. All self-report data was collected using Compusense® – a web-based data collection software.

For this study, we used an in-context, randomized, counterbalanced design with an unscented control. Participants applied the face cream at bedtime in their natural sleep environment. The study lasted Sunday night through Friday morning for 3 consecutive weeks (Table1).

Participants used a different sample each week where the control sample was always used during the second week and the two actives were counterbalanced across participants. Table 1 shows an overview of the design. For each night of the study, participants completed daily questionnaires and tracked their sleep using the SleepScore Max device.

	Participant Cohort	Sample Name
Week 1	Cohort A (N=16)	Active
Week 2	Cohort A (N=32)	Placebo
Week 3	Cohort A (N=16)	Active

Table 1: *Overview of the study design*

Analysis:

For sleep data, objective nightly sleep data was analyzed using JMP Pro (version 15) statistical software using mixed model, with nights nested within participants. An alpha level of 0.05 was used for all statistical tests. The primary comparison was control condition vs. active condition. Percentage changes were calculated as changes from the control condition.

Statistical methods:

Obtained data and percentage variations were submitted to two-way Student t-tests for paired data. The statistical significance value is $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

***Ex-vivo* study**

Skin explants:

Skin organ cultures were prepared from tissues samples derived from abdominal tissue of a 46 years-old Caucasian woman with a phototype II-III (according to Fitzpatrick skin color classification) undergoing routine therapeutic procedures, with donor's consent. The explants were placed in survival in BEM (BIO-EC's Explants Medium) culture medium at 37 °C under 5 % CO2 and 95 % humidity.

Chemical stimulation of human microdissected skin:

After 2 days in a medium culture, the explant was stressed, or not, using a UV simulator Vibert Lourmat RMX 3W with a dose of 13.5 J/cm2 of UVA and a dose of 0.15 J/cm2 of UVB before adding the active at 1% or the placebo. UV was used in this model to mimic daily aggression. After 24h, explants were fixed in RNAlater for genomic study or in formalin solution for histological processing.

Genomic analysis:

The RNAs were extracted from explants using Promega's ReliaPrep™ RNA Tissue Miniprep System (fibrous) before being reverse transcribed (RT) into cDNA with iScript (Bio-Rad). Genes expressions were then determined. For quantification, the number of cycles was normalized according to the reference gene B2M. A gene was considered induced if its expression showed an significant increase greater than or equal to 1.5 compared to the control (fold-change $\geq$1.5). Likewise, a gene was considered repressed if it showed a significant reduction of expression relative to the control (fold-change $\leq$0.65). For each gene of interest, values were calculated between treatment samples in triplicate compared to the matched explant control samples in triplicate for each kinetic time point.

Immunostaining analysis:

Samples were dehydrated and impregnated in paraffin using a Leica PEARL dehydration automat. The samples were embedded using a Leica EG 1160 embedding station. 5-µm-thick sections were made using a Leica RM 2125 Minot-type microtome, and the sections were mounted on Superfrost® histological glass slides. The frozen samples were cut into 7-µm-thick sections using a Leica CM 3050 cryostat. Sections were then mounted on Superfrost® plus silanized glass slides. The microscopical observations were realized using a Leica DMLB, an Olympus BX63 microscope. Pictures were digitized with a numeric DP74 Olympus camera with cellSens storing software. Immunostaining was performed on FFPE skin sections with a monoclonal anti-OGG-1 antibody (Invitrogen, ref. PA1-31402, 1:600) or Catalase antibody (Novus biologicals, ref. NBP2-00492, 1:200) and incubated for 1 hour at room temperature using a Vectastain Kit Vector amplifier system avidin/biotin, and revealed by VIP, a substrate of peroxidase giving a violet signal once oxidized (Vector laboratories, Ref. SK-4600). Intensity of staining in the all epidermidis and the stratum

corneum (L*a*b*- measurement) was determined by image analysis. The production of each marker is quantified by the stained intensity for each treatment and is compared to the stressed condition. Image processing was performed using Fiji software.

Statistical analysis

Obtained data and percentage variations were submitted to two-ways of Student T-test for paired data. The statistical significance value is $p<0.05$ (*p<0.05, **p<0.01, ***p<0.001).

Results.

The active ingredient improves sleep quality

Based on the different parameters analyzed by the device, the essential oil at 0.0025%, showed an improvement of overall sleep parameters compared to the placebo (Figure 1A). More specifically, the active significantly increased total sleep duration ($p<0.05$) as compared to the control condition, which we primarily attributed to a significant increase in Deep Sleep duration ($p<0.05$). Participants on average slept for 6.5 hours (392 minutes) with unscented control as compared to 6.7 hours (402 minutes) with the active (Figure 1B). The deep sleep duration also increased from 1.2 hours (73 minutes) with unscented control to 1.3 hours (79 minutes) with the active (Figure 1C). This increase also resulted in a significant increase in the Body Score (2-point change, $p<0.05$) (data not shown) suggesting more restful sleep in the presence of essential oil. There were no consistent differences between age groups or gender groups.

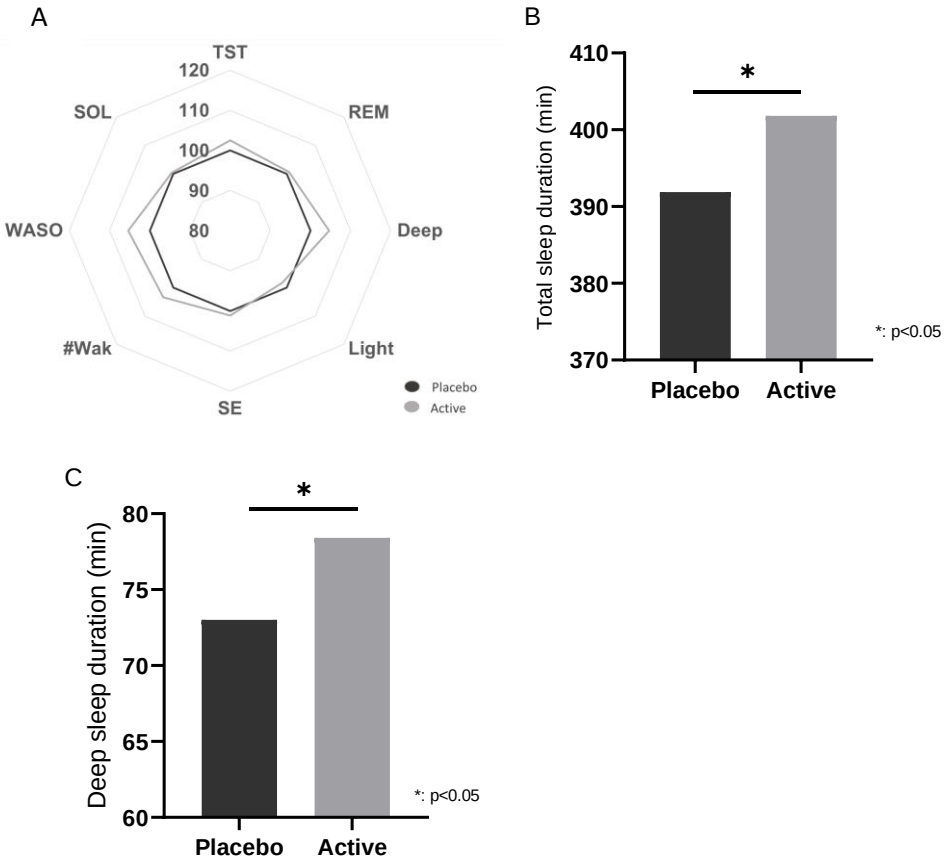

Figure 1: Effects of the Essential oil at 1% (in grey) and the placebo (in black) for objective sleep parameters (A) Overall effect for Active (1%) shown as percentage change from unscented control. TST: Total Sleep Time; REM: REM Sleep Time; Deep: Deep Sleep Time; Light: Light Sleep Time; SE: Sleep Efficiency; #Wak: # of interruptions; WASO: Wake after sleep onset; SOL: Onset Latency. (B) The effect of the EO on Total Sleep Duration and, (C) Deep Sleep Duration in minutes. Data are presented as means ± SEM, *($p < 0.05$) indicate significant differences relative to the unscented control condition

The fact that our active increase deep sleep, a stage essential for body regeneration, led us to study the regenerative effect of our active on skin after daily aggressions.

The ingredient improves skin regeneration

To study the potential repairing effect of the active in skin response to daily stress, a skin *ex-vivo* stressed model was designed. First, the capacity of the active to act on melatonin pathway was studied through different markers such as ASMTL, RORA, SIRT3, and SLC15A1 (PEPT1). Then, expression of different markers modulated by the melatonin were

measured such as markers of inflammation (IL6 and COX-2), oxidation (CAT, GPX1) or DNA repairing (OGG1) as well as collagen (COL1A1, COL3A1).

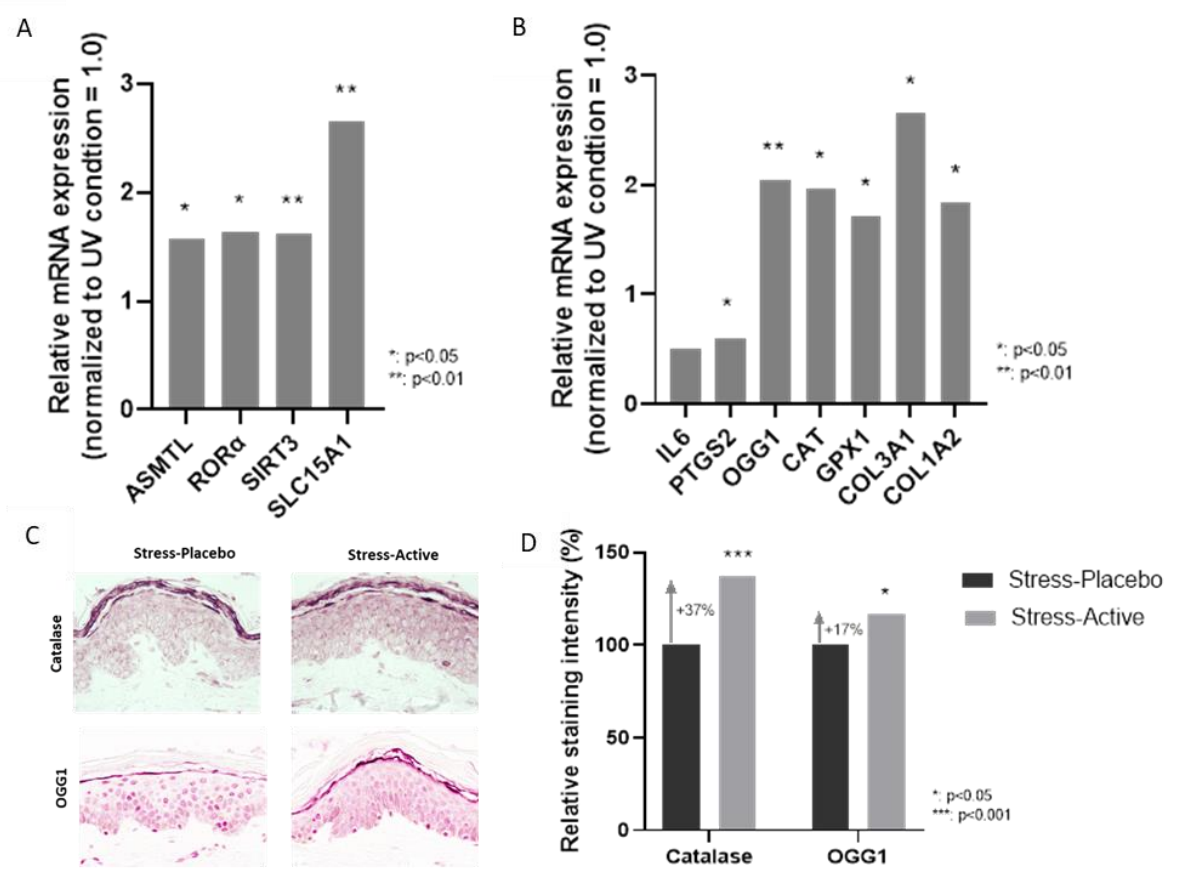

Figure 2: Effect of the active (1%) on stressed markers versus stressed condition in skin explants. (A) Expression of mRNA of melatonin-associated gene expression. (B) Expression of mRNA of stress markers from melatonin pathway. (C) Protein staining and (D) relative intensity for Catalase and OGG1. Data are presented as mean ±SEM values. Statistical analysis was performed using t.test (* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$) to compare each group.

Compared to stressed condition with placebo, the essential oil induced an increase of the expression of various melatonin-associated genes (Figure 2A). Fold changes are: 1.55 (eq. +55%, *p<0.05), 1.63 (eq. +63%, *p<0.05), 1.62 (eq. +62%, **p<0.01) and 2.62 (eq. +162%, **p<0.01) for ASMTL, RORA, SIRT3 and SLC15A1, respectively. In addition, a repression of IL6 and PTGS2 (COX-2) with the active was observed with fold change values of 0.51 (eq. -49%, *p<0.05) and 0.60 (-40%, *p<0.05), respectively (Figure 2B). In a similar context, the essential oil triggered a strong induction of OGG1 with a fold change of 2.02 (eq. +102%,

p<0.01) that is involved in the DNA repair. Moreover, this product induces a strong induction of two antioxidant enzymes. Fold changes are: 1.94 and 1.70 for CAT (eq. +94%*p<0.05) and GPX1 (eq. +70%, *p<0.05), respectively. An effect on COL3A1 and COL1A2, with a fold change of 1.97 (eq. +97%, *p<0.05) and 2.56 (eq. +156%, *p<0.05), tends to prove that the active also acts on the extracellular matrix with an increase of the collagen synthesis. These results were confirmed at the protein level for 2 markers (Figure 2C). An increase of CAT and OGG1 were found with +37% (*p<0.001) and +17% (*p<0.05), respectively.

All these results show the potential of the active offer healing properties and therefore regenerate the skin after daily aggressions.

Discussion.

Sleep disturbances have become a pervasive and prominent problem in the modern 24-h society. In addition to the effect on the physical and mental well-being, poor sleep also impacts our skin quality. Indeed, sleep is an important physiological mechanism to fight skin aging through the regulation of systemic inflammation, oxidative stress or DNA damage. In this study, we presented a novel strategy using a dual approach combining *in-vivo* and *ex-vivo* screening, to identify novel materials able to counteract the sign of skin aging due to sleep disturbance. Based on the well-known potential of volatile compounds in aromatherapy, we explored the capacity of a single EO to improve the sleep quality while having a direct effect on skin. This approach has never been investigated to our knowledge where researchers generally focus on sleep or on skin activity.

First, results from the present study demonstrate the capacity of the selected EO, used alone at a low dosage in a face cream without a perceptible odor, to improve overall sleep quality relative to an unscented control condition. We observed that EO improved the time spent in deep sleep stage during the night. Deep sleep is crucial for physical renewal or hormones release. Insufficient deep sleep can increase the likelihood of a person getting sick, feel depressed, and gain an unhealthy amount of weight [26]. Furthermore, studies have shown that adults with a shorter duration of REM and deep sleep had poorer cognitive and memory performance the next day [27]. Moreover, since deep sleep diminishes with aging, discovery

of ways to safely support and prolong this sleep stage in elders could be of great utility. This effect confirmed the result obtained by Ko *et al*. [21] with the Lavender aroma, the main essential oil studied, although they used essential oils with perceptible odor. Indeed, they showed that the time percentage of deep sleep in the presence of a specific EOs was significantly higher than that for the unscented control night. However, in contrast to our results, Ko *et al*. observed that participants slept for roughly the same time with or without lavender aroma. Here, the active showed its capacity to increase the total sleep time by 10 min each night, or in other words, participants on an average slept an additional 1.25 to 1.75 hours every week. This increase is significant as melatonin showed between 8 and 13min improvement [28] while sedative hypnotics tend to 25min improvement [29].

The effect of the EO was also measured on skin explant with the aim of investigating its regenerative effect after daily aggression. Indeed, it is well known that sleep deprivation induces consequences on skin. Moreover, the fact that deep sleep is the regenerative sleep, we focused on skin regenerative effect. We also looked at the melatonin pathway since this hormone is mainly produce during deep sleep stage and is well known to have positive effect on skin. Indeed, melatonin is synthesized in the skin and present skin effects *via* receptors activation or receptor-independent mechanism [30,31]. The active showed its capacity to modulate the same targets as the melatonin (RORα, SIRT3). It may also increase its synthesis by increasing ASMTL expression as well as its transport to mitochondria via SLC15A1 (PEPT1) modulation. Indeed, ASMTL is an enzyme which catalyzes the final reaction in melatonin biosynthesis, converting Normelatonin to melatonin [32] while oligopeptide transporters PEPT1 allows melatonin to presumably enters mitochondria to have mitochondria-targeted antioxidant effect [33]. Consequently, the EO exhibited anti-inflammatory and antioxidant properties through the increase of antioxidant enzyme, against stress condition. This result is not surprising since EOs are derived from plants and contain a high diversity of volatiles (e.g. terpenes, sesquiterpenes), aromatic (e.g. phenol), and low-molecular-weight compounds that have been widely investigated for cosmetic and pharmaceutical purposes. Several oils such as the Bergamot oil was proved to inhibit IL1-α levels on sebocytes [34]. Cinnamomum camphora (L.) J.Presl essential oil has also been demonstrated to decrease various interleukins (IL6, TNFα or IL-1β) as well as the Alpinia

calcarata Rosc. [35]. Other studies made on Carvacrol, Anethole or linalool for example showed the capacity of these molecules to reduce inflammatory biomarkers through an increase of antioxidant enzyme SOD, CAT, MDA or GSH [36] as we showed with the active. Results from the current study further highlight the potential benefits of a specific EO to protect against environmental stressors on skin that induce the appearance of fatigue and aging. Therefore, the ability of the active to act on both sleep and skin suggest this ingredient can decrease visible sign of skin fatigue. A clinical study to validate this hypothesis is currently ongoing.

Conclusion

All together we have developed a new multitargets strategy to identify an EO with improved sleep deficiency-related signs on skin. This single material used as a cosmetic ingredient at very low concentration without smell perception, showed for the first time to our knowledge, an impact on volunteers sleep quality using in-context biometric measures. The additional *ex-vivo* effects obtained on skin further demonstrate the potential to counteract key skin indicators of sleep deprivation, thanks to the activation of the melatonin pathway, and make this ingredient an excellent candidate to repair facial signs of fatigue and skin aging. Ongoing clinical trial is now needed to confirm these results on volunteer' skin.

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Conflict of Interest Statement

NONE

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