

Article

Measurement of Stress Relief during Scented Cosmetic Product Application Using a Mood Questionnaire, Stress Hormone Levels and Brain Activation

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Abstract: Nowadays, consumers' well-being plays a decisive role in the purchase of cosmetic products. Although factors influencing consumers' well-being are very subjective, companies strive to develop their products in such a way that a positive effect is likely. Therefore, methods are required to objectively explore and scientifically prove the product's performance on humans. In this placebo-controlled study, a method was developed to evaluate relaxation or stress relief associated with one olfactory ingredient of a cosmetic product (face cream). Our experimental protocol included product testing in 25 healthy females, while an emotion questionnaire, analysis of saliva samples regarding the concentration of the hormones cortisol and α -amylase and mobile EEG measurement for quantification of the alpha brain waves before and after stress induction were conducted. It was shown that with this experimental design, the sample with the ingredient produced significant stress relief, as evidenced by significantly less negative emotion, significantly lowered cortisol levels and showed a trend towards a significant increase in alpha activity compared to placebo application. Our data provide evidence that this method is suitable for analyzing the differences between the two samples. In the future, this method can be utilized in the current or a further optimized form to evaluate the psychophysiological effects of cosmetic products on humans.

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Keywords: aroma; fragrance; scent; skin care; positive mood; relaxation; cortisol; amylase; well-being; EEG; neuroscience; brain activity; alpha waves

1. Introduction

Especially in times of crisis, such as the pandemic, the general stress level of consumers increases [1,2]. To relax, many people enjoy wellness experiences using cosmetic products such as oils, bubble baths and scented shower gels or body lotions, especially with stress relieving properties communicated via marketing claims [3–5]. Whether the relaxing effect is caused by the product or the relaxing environment is not always clear. Therefore, consumers, authorities and companies along the value chain request better scientific evidence of the effects of cosmetic products on humans [6,7].

Aroma components of natural products have been used for mental, spiritual and physical healing since the beginning of recorded history. The best-known relaxing fragrance, lavender, has already been shown to induce a calming effect in numerous studies; for example, salivary stress markers are reduced [8], sleep quality is enhanced [9] and anxiety is reduced [10], to name just a few out of numerous examples. The aromatic components of the relatively unknown plant *Myrothamnus flabellifolia* can also have a

relaxing effect on humans. These shrubs can resurrect in alternating humidity conditions after almost complete desiccation. Extracts from this plant have already been proposed in the literature for use in cosmetic products, for example, due to polyphenols [11–13], their healing [14] or their fibroblast-activating effect [15]. Their antimicrobial and antiviral effect was also proven [16,17]. *Myrothamnus flabellifolia* leaves produce resins and essential oils whose main components are *trans*-pinocarveol and pinocarvone, which have, respectively, a tart and minty odor [14,16,18]. Other aroma components identified include α -pinene, limonene, linalool and β -selinene [14,19]. Kessler et al. (2014) showed that pinocarveol is a potent modifier of GABA_A receptor function [20]. Since the GABAergic system is highly involved in the regulation of anxiety, this could be a possible explanation for the anxiolytic effect of this aroma component [21,22]. It was further established that the stimulation of the receptors in the nose through fragrance inhalation exerts various psychophysiological effects on human beings [23,24], including effects on mood [25] or hormone levels [26]. For a detailed systematic review of psychophysiological responses to odors, see Loos et al. [27].

The psychophysiological reaction to an odor can be measured via EEG recordings placed on the surface of the scalp [28,29]. Different studies have demonstrated the applicability of EEG measurements to study the influence of scents on brain activity [30,31].

The EEG power spectrum is classified into different frequency bands such as gamma (30–44 Hz), beta (13–30 Hz), alpha (7.5–13 Hz), theta (4–8 Hz) and delta (1–4 Hz) frequency bands, and each band is correlated with different features of brain states. In the common awake state with eyes open, gamma and beta waves are dominant. When dozing or in a state of relaxation, alpha activity increases [32]. Theta waves occur during the transition from the conscious state toward sleepiness and are attributed to access to unconscious material, creative inspiration and deep meditation [33]. During deep sleep, delta waves have a high proportion [31]. In particular, alpha waves are associated with mental coordination, calmness, integration and learning states of the brain [34–36]. An increase in alpha activity is highly correlated with a comfortable state in humans after inhaling lavender oil [37] and a relaxed state after exposure to neroli and grapefruit oil [38]. Sowndhararajan and Kim (2016) have already provided a comprehensive overview of previous studies in the field [39].

Measurement with conventional EEG shows numerous disadvantages, such as the high costs of the devices, the time-consuming attachment of the electrodes and the distraction of the subjects due to the cables and attached electrodes. Further, the association with medical equipment could trigger negative effects in the participants, which may not be transferable to a relaxing and mindful practice and could possibly falsify the results. Therefore, an alternative method of measuring brain activity is required in which these negative effects are reduced, and the benefits of the measurement method are maintained. One possibility would be to use a mobile EEG device with four electrodes that is significantly less expensive, is applied in a time-saving manner similar to a headband and is more comfortable and less invasive for the test person. Previous studies showed good applicability of mobile EEG devices regarding psychophysiological response measurements, but in those studies, mobile devices with more than 4, in particular, 14 electrodes, were utilized [40]. Further, with the help of those devices, brain response in a sports science context [41], in urban environments [42] or pathological processes such as epilepsy were monitored [43]. To date, within two publications, the scientific use of the Muse-portable EEG headband device with four electrodes was introduced [44,45]. Further, the system was used for the investigation of visual attention [46] or emotional well-being [47] but also for prediction of stroke severity [48]. However, due to their affordability, fast attachment and high wearing comfort, mobile EEGs have also been offered outside the scientific community as part of neurofeedback applications to improve sleep [43], concentration [49] or meditation [50,51], which proves the practicability of the system. Recently, it was confirmed that the emotional effect of cosmetics can be investigated using

EEG and that stimuli arising from cosmetics can have an influence on the human brain [52,53]. In a previous study, the psychophysiological effects of cosmetics were shown [54], although other measuring methods were used, such as heartbeat and skin conductance measurements [55,56], facial electromyogram and mood questionnaires [57,58]. To the best of the authors' knowledge, no literature is cited to date describing the use of a validated and standardized method for exploration of the psychophysiological effects of cosmetic products or ingredients using a mobile four-electrode EEG system.

Another stress marker is the production of stress hormones, e.g., cortisol and α -amylase. As Chrousos (2009) stated in a review, the main peripheral effectors of the stress system are glucocorticoids regulated by the hypothalamic–pituitary–adrenal (HPA) axis, and the catecholamines are norepinephrines and epinephrines regulated by the systemic and adrenomedullary sympathetic nervous systems [59]. Thus, through stress, the HPA axis is activated, and corticotropin-releasing hormones and adrenocorticotrophic hormones are released, leading to an increased level of glucocorticoid cortisol. The analysis of salivary cortisol as a stress biomarker offers significant advantages over the analysis of blood cortisol, as the sample collection is non-invasive and more time- and cost-effective. The concentration of cortisol in saliva provides a valid and reliable reflection of unbound cortisol in blood [60]. Another biomarker of stress is the release of salivary α -amylase through the activation of the autonomic nervous system [61]. Previous research findings show a clear relationship between experiencing different stress situations and increasing salivary α -amylase levels [62,63]. Takai et al. (2004) examined both cortisol and α -amylase concentrations in saliva during stress induced by an unpleasant video and showed that α -amylase concentrations increased shortly after the onset of stress induction, while cortisol concentrations increased with a slight delay [64].

The psychological effect of an odor can also be perceived by the human subjects themselves and can be quantified using a questionnaire. For this purpose, various scales exist. The Positive and Negative Affect Schedule (PANAS) provides a way to briefly and easily determine the state of mind of individuals and examine positive (PA) and negative (NA) affect, each with 10 items. A high PA is a measure of a person's enthusiasm, activity and alertness, while a low PA indicates sadness and lethargy. A high NA represents distress and unpleasurable engagement, while a low NA reflects calmness and serenity [65]. Yim et al. (2010) combined cortisol level measurement and PANAS after standardized stress induction in adults and children [66]. They showed stress increases salivary cortisol, lowers positive affect and raises negative affect. However, no correlation was found between the self-reported mood state (PA and NA) and the change in cortisol level. Another study found a correlation between a dampening of the cortisol rise, a reduction of state anxiety, a favorable change in mood profile and a reduction of non-verbal behavioral patterns that signal anxiety, motivational conflict and avoidance while using a cosmetic product [52]. Although research on stress reduction in relation to use of cosmetics or perfumed products is still in its early stages, recent results show that the application of a cosmetic cream enriched with essential oils should be considered as a stress resilience fostering strategy [52,67].

Already existing data indicate that the applicability of the methods for the evaluation of cosmetics and fragrances for the measurement of a psychophysiological effect is likely [52], but concrete evidence is missing. Therefore, we see a need to elaborate on this aspect with a reliable and reproducible experimental approach. Consequently, the aim of this study was to develop a method combining the PANAS questionnaire, mobile EEG measurement as well as analysis of saliva samples regarding the concentration of cortisol and α -amylase before and after experimental stress induction and to prove its applicability in a use case with and without an active ingredient. This research will enable a reliable standard for the objective scientific examination of the effectiveness of cosmetic products or ingredients in the future.

2. Materials and Methods

2.1. Sensory Evaluation of the Active Ingredient

To prove that a sensory perception of the active ingredient takes place, the fragrance was characterized by a trained sensory panel. The active ingredient was a *Myrothamnus flabellifolia* CO₂ extract. Branches of the bush were harvested in wild pick in accordance with the South African Access and Benefit Sharing Regulations under the Nagoya Protocol. The project will support local communities and sustain the population of *Myrothamnus flabellifolia* bushes. The resulting oily extract was diluted in neutral oil (Caprylic/Capric Triglyceride) to standardize the extract to a concentration of 11.4% essential oils. However, it should be noted that the extract is a natural product with fluctuating content of essential oils. Due to the different growth conditions of each batch, the final content may vary. This extract was evaluated using a conventional aroma profile analysis by 12 trained testers according to DIN-Norm [Error! Reference source not found.]. In the first step, a collection of descriptive attribute characteristic of the sample was created. From these attributes, the most characteristic five attributes were selected, and similar ones were summarized. In the second step, an intensity rating of the sample was determined with respect to the five selected attributes on a 10-point scale. We determined the mean and standard deviation for each attribute.

2.2. Method Development for Instrumental Measurement of Stress Relief

To objectively investigate and scientifically prove a cosmetic product's performance on humans, we developed a method to evaluate the relaxation or stress relief associated with the odor of the complete product as well as an assessment of the pleasantness, arousal and emotional state of the consumers.

Ethical approval for this study protocol was obtained from the University Hospital at FAU Erlangen-Nürnberg prior to commencing tests (No. 75_20B). A hygiene concept was implemented specifically for this study in terms of a questionnaire, face masks, disinfection, keeping distance, and airing out the testing room due to the COVID-19 pandemic. The extract was proven safe in a comprehensive Toxicology and Safety Assessment Report on 17 December 2019 conducted at Rahn AG. All other ingredients are commonly used in cosmetic formulations and were evaluated as safe by DERMATEST (Münster, Germany) with a similar formulation on 16 June 2017.

Products with the active compound (AC) and without the active compound (placebo, PL) were randomly tested. Both formulations were perfumed with vanilla scent under the hypothesis that the intrinsic odor of the active ingredient could only be perceived subconsciously. The samples were identical in all cosmetically relevant characteristic parameters. Since there were no perceivable differences between the samples in terms of viscosity, we did not perform rheological tests. The samples were both filled in identical airless pump dispensers, meaning that the application volume of the cream was equal. Therefore, it can be assumed that all measured differences between the two samples were due to the active ingredient and that the matrix had no influence on the test result. Table 1 shows the characterization of the samples.

Table 1. Characterization of the samples (AC = active compound, PL = placebo).

	AC	PL
Ingredients (INCI)	Water, Caprylic/Capric Triglyceride, Glyceryl Stearate Citrate, Cetearyl Alcohol, Phenoxyethanol, Sucrose Stearate, Carbomer, Caprylyl Glycol, Fragrance (Parfum), Xanthan Gum, <i>Myrothamnus Flabellifolia</i> Leaf/Stem Extract, Sodium Hydroxide	Water, Caprylic/Capric Triglyceride, Glyceryl Stearate Citrate, Cetearyl Alcohol, Phenoxyethanol, Sucrose Stearate, Carbomer, Caprylyl Glycol, Fragrance (Parfum), Xanthan Gum, Sodium Hydroxide
Active Compound	3.0% <i>Myrothamnus flabellifolia</i> CO ₂ -extract	3.0% Water
pH-value	5.8	5.8
Density [g/mL]	0.9–1.0	0.9–1.0
Production date	19 December 2019	19 December 2019
Shelf life [months]	36	36

The two samples were coded and tested in 25 healthy women. The subjects were unaware of the sample they were receiving. Each subject received both samples on two different days, with the order of samples pseudorandomized across subjects. The subjects were between 18 and 50 years old (mean: 25.7 years, SD: 4.1 years), reported normal taste and smell sensitivity, normal or corrected to normal vision, had no history of neurological or mental diseases, were not pregnant, not breastfeeding, not using drugs and had no symptoms of a SARS-CoV-2 infection. Four out of twenty-five subjects were smokers. The mental condition of the participants was examined using the Beck Depression Inventory (BDI, mean: 3.0, SD: 3.3, range: 0–14) and the Mini Mental State Examination (MMSE, mean: 29.6, SD: 0.7, range: 28–30). Odor identification performance was investigated using the Sniffin' Sticks identification test (mean: 14.1, SD: 0.9, range: 12–16).

The implementation of the method included a stress induction block and a product testing block. As a preparatory step, the EEG band was adapted to the subject's head and calibrated for 5 min. These data were only used to verify the functionality of the experimental setup and were not evaluated. In the stress induction block, stress was induced using images of the OASIS image database [68] over a period of 2.5 min. The images were chosen to be negative and of high emotional arousal. Two image sets with identical mean negativity and arousal levels were created, one for each testing session. After stress induction, brain responses were measured over a period of 5 min [70,71]. After this time, a saliva sample was obtained for the examination of α -amylase and cortisol [61,64], and the participant's mood state was evaluated by the PANAS questionnaire [65,72]. Afterwards, in the odor testing block, the subjects applied a defined amount of the product or placebo to their cheeks and sensed with their eyes closed for one minute. Subsequently, we measured brain responses over a period of 5 min, and a saliva sample and a mood questionnaire were obtained and conducted again. The scheme of the experimental paradigm is shown in Figure 1.

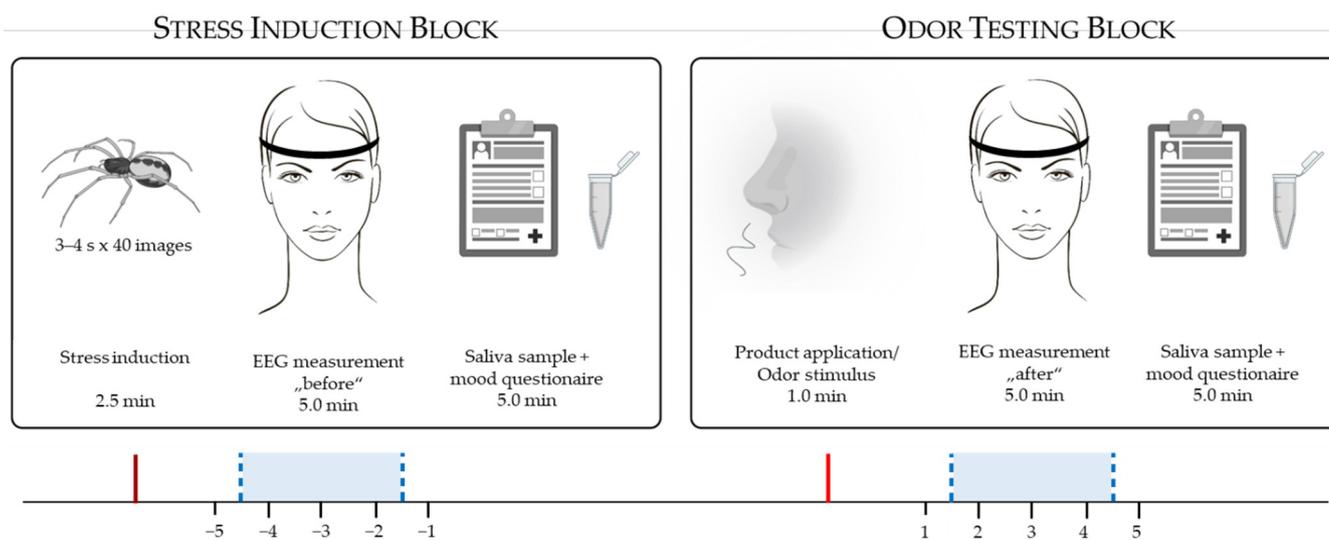


Figure 1. Experimental paradigm for stress induction and product testing phase.

EEG data were acquired with the EEG headband Muse 2 (IntraXon, Toronto, ON, Canada) [70] and processed with Mind Monitor [73] which uses Fast Fourier Transformation to compute the power spectral densities for the alpha, beta, gamma, delta and theta frequency bands and calculates the absolute band powers using the logarithm of the sum of the respective power spectral densities. We averaged the alpha band powers across the two TP electrodes. After 5 min of recording, we cut off the first and last minute to correct for influences of the experimenter and the external surrounding of the participant. Then, we calculated the mean of the resulting three minutes per subject and subsession. To examine the effect of sample application on alpha activity, we conducted a rm ANOVA with time (before and after product application) and sample (AC and PL) as factors. Twenty-four subjects were included in the analysis as one subject had to be excluded due to missing electrode contact.

Saliva samples were collected via Salivettes (Sarstedt, Nümbrecht, Germany) and were stored for later assessment at $-30\text{ }^{\circ}\text{C}$ after the laboratory session. They were analyzed at the Chair of Health Psychology at FAU Erlangen. The samples were centrifuged at 2000 g and $4\text{ }^{\circ}\text{C}$ for 10 min. Cortisol concentrations were assessed in duplicate using the chemiluminescence immunoassay (CLIA, IBL International, Hamburg, Germany). The quantification of α -amylase levels was conducted using in-house enzyme kinetic assays, also in duplicate determinations with reagents from Roche Diagnostics (Mannheim, Germany). To examine the effect of AC vs. PL on the concentration of the stress hormones cortisol and α -amylase in the saliva, we performed rm ANOVAs with the factors time (before and after product application) and sample (AC and PL). Due to missing data on hormone concentration, we had to exclude one subject from cortisol analysis and two subjects from the α -amylase analysis.

The PANAS questionnaire consisted of 10 positive and 10 negative items, wherein each item was rated from 1 (very slightly or not at all) to 5 (extremely) [65]. For a detailed description, please refer to Breyer and Blume (2016) [72]. The Positive Affect Score (sum of all positive items) and the Negative Affect Score (sum of all negative items) were calculated for each subject. Both scores ranged from a minimum of 5 (low affect) to 50 (high affect). Thus, both scores were calculated for each subject before and after product application, analogously for the AC and PL. To examine the effect of AC vs. PL on the positive and negative affect scores of PANAS, we performed rm ANOVAs with the factors time (before and after product application) and sample (AC and PL).

Statistical analyses were conducted in JASP 0.16 [74] and Python 3.7.1 [75]. *p*-values of post hoc tests were adjusted using Holm correction [76].

3. Results

3.1. Sensory Aroma Evaluation

During the sensory aroma evaluation, the attributes acid-like, citrus-like, herb-like (dill-like, thyme-like, rosemary-like), fatty/oily and fir-like/conifer-like/resin-like were identified as most characteristic of the *Myrothamnus flabellifolia* extract. The attributes were rated on a scale from 0 to 10 (mean \pm SD), with fir tree-like (7.25 ± 2.05) as the most dominant attribute, followed by citrus-like (6.00 ± 1.81), herb-like (5.92 ± 2.39), acid-like (3.75 ± 2.05) and fatty/oily (2.83 ± 1.47). These data provide evidence that the extract is orthonasally perceivable and a potential effect is possible. The results are shown in Figure 2.

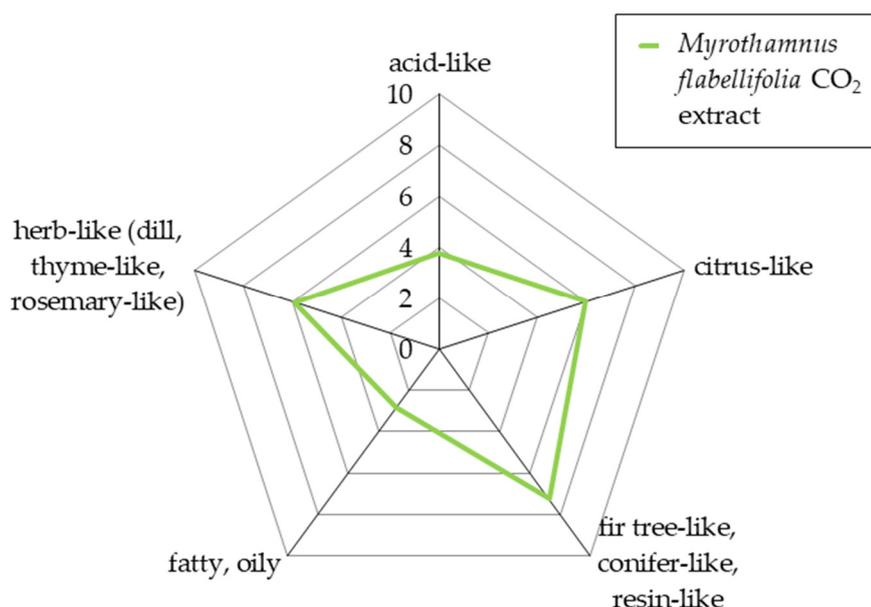


Figure 2. Conventional aroma profile of *Myrothamnus flabellifolia* extract evaluated by 12 trained testers.

3.2. Effects of AC Application on Mood

The evaluation of the mood questionnaire (see Table 2, Figure 3) established a trend towards a significant interaction of time and sample ($p = 0.076$) with an enhanced PA score after AC application (before: 23.9 ± 8.9 , after: 25.7 ± 8.7) in contrast to PL application (before: 24.0 ± 7.3 , after: 23.6 ± 8.0). The main effects were not significant (time: $p = 0.385$, sample: $p = 0.358$). There was no significant interaction concerning the NA score ($p = 0.398$). However, there was a significant main effect of time ($p < 0.001$) due to significantly decreased NA scores after product application of both samples (AC before: 16.0 ± 6.2 , after: 11.2 ± 1.9 ; PL before: 17.2 ± 6.6 , after: 11.4 ± 1.9).

Table 2. Results of rm ANOVA on the positive and negative affect with the factors time (before and after product and placebo application) and sample (AC and PL) (N = 25).

Effect	DF _{num}	DF _{den}	F	p-Value	η_p^2
Positive Affect Score					
Time	1	24	0.783	0.385	0.032
Sample	1	24	0.878	0.358	0.035
Time×Sample	1	24	3.442	0.076 (*)	0.125
Negative Affect Score					
Time	1	24	27.615	<0.001 ***	0.535
Sample	1	24	1.676	0.208	0.065

Time×Sample	1	24	0.741	0.398	0.030
DF _{num} = degrees of freedom in numerator, DF _{den} = degrees of freedom in denominator, F = noncentral F distribution, η_p^2 = effect size, partial Eta square, Note: (*) trend towards statistical significance, *** Significance with $p < 0.001$.					

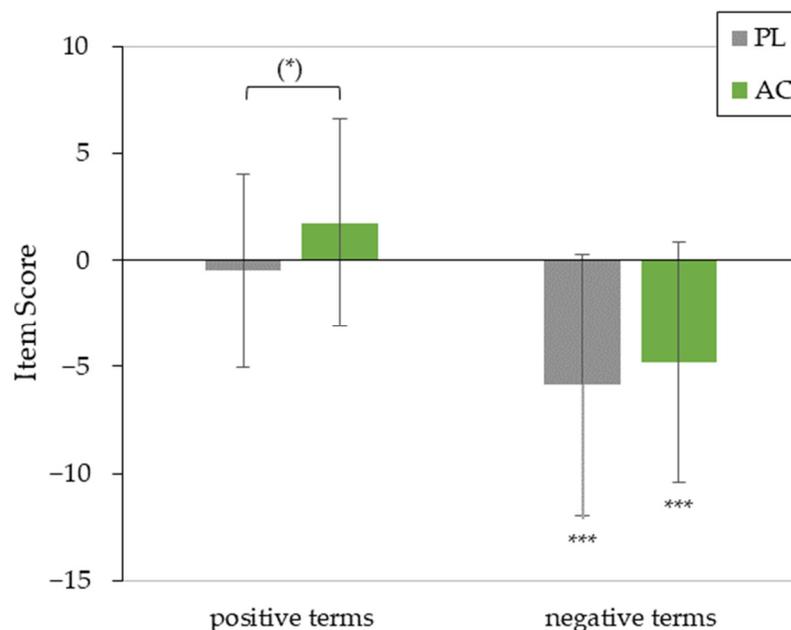


Figure 3. Changes (after versus before product application) for the positive and negative items of the Positive and negative affect schedule (PANAS) for the samples placebo (PL) and active compound (AC) (mean \pm SD). Note: (*) Trend towards statistical significance with $p < 0.1$. *** Significance with $p < 0.001$.

3.3. Effect of AC Application on Stress Hormones

For the biomarker cortisol we established a significant interaction effect of time and sample ($p = 0.017$, Table 3, Figure 4), resulting from a significantly decreased concentration of salivary cortisol after AC application (before: 6.32 ± 4.17 , after: 5.89 ± 3.65 , $p = 0.042$) in contrast to PL application that led to no significant change in cortisol concentration (before: 6.17 ± 4.01 , after: 6.50 ± 3.91 , $p = 1.000$). For α -amylase, there was neither a significant interaction effect ($p = 0.112$) nor significant main effects (time: $p = 0.763$, sample: $p = 0.801$, Table 3).

Table 3. Results of rm ANOVA of cortisol [nmol/L] (N = 24) and α -amylase [U/mL] (N = 23) in saliva with the factors time (before and after product application) and samples (AC and PL).

Effect	DF _{num}	DF _{den}	F	p-Value	η_p^2
Cortisol					
Time	1	23	2.017	0.169	0.081
Sample	1	23	0.049	0.826	0.002
Time×Sample	1	23	6.583	0.017 *	0.223
α -amylase					
Time	1	22	0.093	0.763	0.004
Sample	1	22	0.065	0.801	0.003
Time×Sample	1	22	2.743	0.112	0.111

DF_{num} = degrees of freedom in numerator, DF_{den} = degrees of freedom in denominator, F = noncentral F distribution, η_p^2 = effect size, partial Eta square. Note: * Significance with $p < 0.05$.

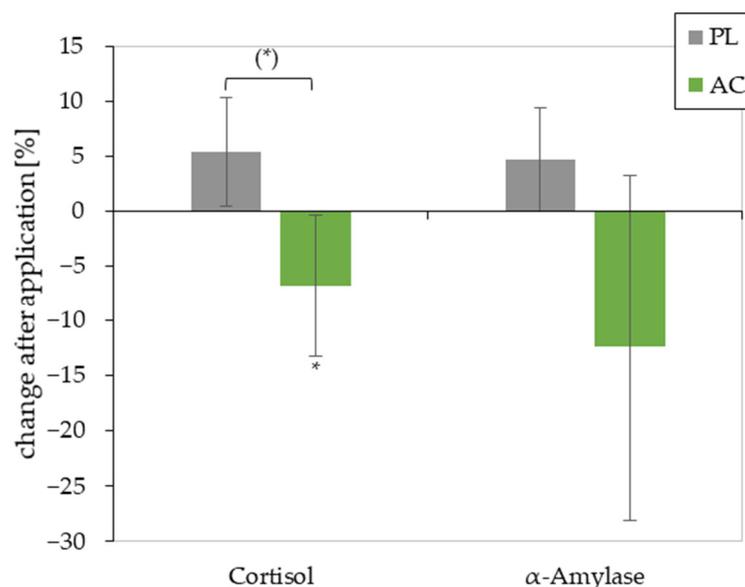


Figure 4. Change of salivary concentration of cortisol and α -amylase when compared after vs. before product application of the samples placebo (PL) and active compound (AC) (mean \pm SD). Note: (*) Trend towards statistical significance with $p < 0.1$. * Significance with $p < 0.05$.

3.4. Effects of AC application on Brain Alpha Waves

Brain activity measured with EEG showed a trend towards a significant interaction effect of time and sample on the alpha-activity in the temporal brain regions (see Table 4) due to higher alpha activity after AC application in contrast to PL application (before: AC 0.886 ± 0.312 , PL 0.910 ± 0.278 ; after: AC 0.881 ± 0.328 , PL 0.861 ± 0.284). The main effects for time and sample were non-significant.

Table 4. Effect of product application on alpha activity. A repeated-measures ANOVA with the factors time (before, after) and sample (AC, PL) was conducted (N = 24).

Effect	DF _{num}	DF _{den}	F	p-Value	η_p^2
Time	1	23	2.261	0.146	0.090
Sample	1	23	0.005	0.944	<0.001
Time×Sample	1	23	3.367	0.079 (*)	0.128

DF_{num} = degrees of freedom in numerator, DF_{den} = degrees of freedom in denominator, F = noncentral F distribution, η_p^2 = effect size, partial Eta square. Note: (*) trend towards statistical significance.

The EEG data depicted in Figure 5 show a curve in which the alpha waves of both placebo and active started at a higher point. This indicates relaxation, although the stress induction took place in direct temporal proximity. It can be assumed that the brain of the subjects needed a certain time to process the stress induction and to react with a change of activity, similar to stress hormones. It can be seen that at -2 min, the alpha value was at its lowest, and thus, the stress level was at its highest, confirming that a waiting time of 5 min was suitable for this study. Afterwards, the application of the product took place. In the progression of the placebo curve, it is visible that from the measuring point at -2 min and the measuring point at $+2$ min, the absolute value changed slightly, therefore the placebo had barely any influence on the test persons. During application of the active compound, alpha activity increased between the time points -2 min to $+2$ min, indicating relaxation due to the application of the cream.

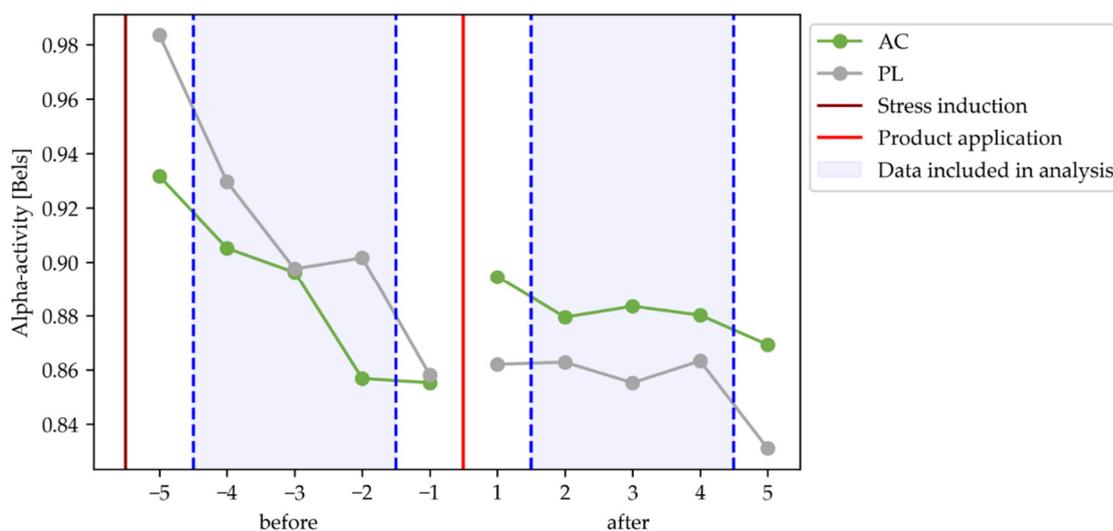


Figure 5. Alpha-activity in the frontal brain regions before and after the product application of the samples active compound (AC) and placebo (PL). Mean data from all subjects (N = 24) were pooled per minute and plotted as dots. The red line represents the product application. For the analysis, the blue areas between minute 2 and minute 4 were included, as this excluded any effects at the start of and towards the end of the measurement. At these times, interaction with the experimenter and the alarm clock took place and could have been an additional stress factor for the subjects.

4. Discussion

The aim of this research was to establish an experimental paradigm to evaluate the stress-relieving effects of a cosmetic product using the combination of a mood questionnaire, salivary stress hormone levels and brain activity.

Sensory aroma evaluation (Section 3.1) describes the major aroma components of the product and is in line with already published data [16,19]. One major compound in the essential oil of *Myrothamnus flabellifolia* is *trans*-pinocaveol, which is associated with a woody–balsamic aroma. Another major component is limonene, which may be responsible for the citrus-like odor [16,77].

Our results of the PANAS analysis (Section 3.2) indicate that overall positive feelings increased among subjects, while negative feelings were reduced when the AC sample was used. As such, the odor of *Myrothamnus flabellifolia* affected subjective reported feelings. Since odors are known for a strong connection to emotions and mood [78,79], it is feasible that the odor of the cosmetic product elicits this effect [80]. Data from the literature confirm that it is possible to measure a positive change in mood profile, as well as a reduction in anxiety and nonverbal avoidance behaviors, after using a specific cosmetic product containing essential oils [52].

Salivary stress hormone analysis (Section 3.3) indicated a clear increase in both α -amylase and cortisol levels after product application during PL application, while a clear decrease was visible during AC application compared with the data before application. It was previously shown that applying stress significantly increases the concentrations of the stress hormones cortisol and α -amylase [62–64]. On the other hand, stress hormones can be reduced or less increased by special therapies [81,82], including odor stimulation [83] or essential oil inhalation [84–86]. Our data indicate that stress hormone levels in the subjects decreased with the use of the AC sample. Therefore, the odor of essential oils in cosmetic products can also positively influence stress hormone concentrations.

The brain activation measurements (Section 3.4) showed a trend towards a significant increase in alpha activity after odor exposure to the AC sample containing the extract after an induction phase. In the literature, this effect was also described by the inhalation of other extracts and essential oils [38–40].

Based on the data of this use case, the application of the formulation with the active compound, namely essential oils of *Myrothamnus flabellifolia*, caused a considerable trend toward a significantly more positive mood, a significant decrease in the level of the stress hormone cortisol and a statistical trend for higher alpha activity in the temporal regions compared to the placebo. *Myrothamnus flabellifolia* is used in traditional medicine, for example, in Madagascar, to treat respiratory diseases, kidney problems, asthma or herpes simplex virus type 1, amongst others. The essential oil contains biologically active compounds with antiviral, antimicrobial and fungicidal activity [16,17,20]. We were able to show that the essential oil also has a psychophysiological effect, namely a stress-relieving effect, that can be achieved if it is used in cosmetic products, particularly in face creams. These properties, in addition to other characteristics such as the presence of polyphenols [11–13] or a fibroblast-activating effect [15], make the essential oil of *Myrothamnus flabellifolia* a promising cosmetic ingredient.

Overall, the evaluation of the values of PANAS, the salivary hormone concentrations and the brain waves with the EEG headband showed high interpretability of the results. Thus, it could be proven that the study design, the method including the mobile EEG measurement coupled with the stress hormone analysis and the evaluation of the mood questionnaire were suitable to investigate the performance of two cosmetic products in comparison.

However, there are a few limitations of the current study. Stress induction was performed using pictures from various stressful life situations [68]. Nevertheless, the stress response to real situations might be different and other stress factors were not tested. The exposure of the subjects to the product took place in a calm and concentration-enhancing environment, which could differ from the practice and daily routines of consumers.

A complete calibration of the measured effects with the concentration of the active ingredient was not yet possible with the data. Therefore, the measured effect can only be confirmed for the used concentration of 3% *Myrothamnus flabellifolia* extract. It should be taken into account that the percentage of essential oils in the extract can vary between 10 and 15% and, accordingly, at a feed concentration of 3%, can range from 0.3 g to 0.45 g per 100 g of product. It is recommended to adjust the input concentration of the extract to the essential oil content of the respective batch or to standardize the extract. Additionally, the effect can only be confirmed for the batch tested, as various metabolites may be produced by different factors during plant growth [87].

Furthermore, one difference between the AC and PL variants, apart from the active content, was the content of water and triglycerides, respectively. Since the extract was diluted with triglycerides, the main component of the 3% activity extract was a component of the oil phase. In the PL sample, this 3% oil phase was absent, and the water phase was increased by 3%. This could have an effect on viscosity and spreadability, and thus overall skin feel [88]. The authors are not aware of any data describing the influence of a small deviation of the emulsion phases of approx. 3%. For future work, we recommend quantifying skin feel / tactile perception using both physical and psychological methods and enabling predictability through correlation with ingredients in the formulation. Additionally, the altered polarity due to the oil phase difference could also change the perception of the aroma components. However, since essential oils are more soluble in the oil phase, increasing the oil phase would hinder their liberation [89]. According to the knowledge of the authors, no study was found in the literature describing the impact of such a small change in the oil phase on human perception. In addition to the proportion of the oil phase, the viscosity, the type and amount of emulsifiers and the composition of the oil phase, e.g. unsaturated fatty acids or the chain length of the emollients, may also play a role in the perception of the scents. This can also be investigated in future work. Based on current experience, we recommend balancing the actives in the placebo with an ingredient of similar polarity for further studies.

Additionally, the vanilla scent was used to mask the essential oil in both variants AC and PL. While this provides comparability, the effect of the extract demonstrated in this approach could be a synergy between vanilla fragrance and the essential oil components [90]. Even though research on synergistic fragrance components in cosmetics is still in its early stages, this could be investigated more deeply in future work.

While this entire method can be applied to support claims about well-being and consumer acceptance of active ingredients or products, the effectiveness of each individual cosmetic product must be scientifically proven. As the number of study participants in this study was rather low and significant effects were not established for all of the investigated parameters, additional proof on finished cosmetic products with the active ingredient is advised. We recommend a panel size of 25 or larger for similar studies. Further studies need to be conducted to explore the transferability of the results to real life or other product categories. For example, the authors suggest investigating whether these or improved methods can be applied to rinse-off cosmetics such as shower gels or bath products. These products present challenges as they spend less time directly on the skin than leave-on products, yet the fragrances are more diffused in the air due to contact with warm water or steam. This could lead to dilution effects, but also to an increase in volatility.

Regardless of the used product, the proven effects can only be achieved if the application conditions of this study are followed exactly. Therefore, conditions to achieve the proved effectiveness should be stated on the label to guarantee correct use of the product according to the claims regulation currently in force. Additionally, it is recommended to determine the quantitative connection between signal intensity of the measurements and well-being in future research. This will provide a dose-response relationship between active ingredient concentration and the psychophysiological effects and enable the use of the ingredient in other concentrations.

5. Conclusions

The use of odors in cosmetic products to support consumer well-being is becoming increasingly popular, while at the same time, higher scientific standards are required to demonstrate effectiveness. Therefore, a method was developed combining stress induction with measurement of alpha waves of the brain, stress biomarkers in the saliva and mood using a questionnaire before and after exposure to products with and without active ingredients. The method was proved suitable for documenting the stress relieving effect of an active ingredient in a cosmetic product compared to a placebo. Significant results were achieved in this use case for some parameters. Further work is necessary to improve the transferability to practice.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of University Hospital at FAU Erlangen-Nürnberg (protocol code 75_20B, date of approval 19 October 2020).

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