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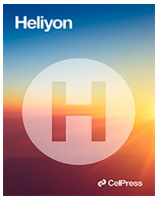

Research article

Exploring the physiological response differences of β-caryophyllene, linalool and citral inhalation and their anxiolytic potential

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ABSTRACT

Essential oils with β-caryophyllene, citral, and linalool as key compounds often exhibit some anti-anxiety like effects in aromatherapy. However, evidence of the effect of these three compounds through human inhalation remains limited. It is worth exploring their potential anxiolytic effect through the olfactory pathway, and finding out whether the three compounds lead to different physiological responses. A total of 48 subjects were randomly assigned to three odor (β-caryophyllene, citral, and linalool) inhalation groups and one control (odorless jojoba oil) group. Stress stimulation was induced using n-back and mental arithmetic tasks. The odor was administered before the task test session. Assessments including the State-Trait Anxiety Inventory (STAI), electroencephalogram (EEG) activities, facial expressions, several physiological indicators, and a self-report scale of subjective perception of the odor environments were carried out. The changes before and after inhalation, as well as the inter-group differences, were analyzed. Both β-caryophyllene and citral inhalation led to a significant decrease in anxiety levels, while only β-caryophyllene resulted in a notable reduction across both sub-scales of STAI. Following the odor inhalation, heart rate significantly decreased in all three groups, with the β-caryophyllene group exhibiting the most pronounced decline. While the systolic blood pressure of the linalool group demonstrated a statistically significant difference. Regarding facial expressions, β-caryophyllene significantly increased the ratio of 'Happiness' and decreased the ratio of 'Fear'. In the non-task state, citral reduced the power of frontal alpha, delta, and theta waves while β-caryophyllene had a similar effect. All odor inhalation groups showed increased delta and theta waves after the task compared with the control group, with the β-caryophyllene group having notably lower frontal beta waves. β-Caryophyllene and citral exhibited good anti-anxiety effects. Subjects receiving different odors showed different EEG and physiological responses, indicating the differences in emotional regulation ways among the three compounds.

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1. Introduction

Anxiety is a prevalent psychological phenomenon, moderate anxiety could increase motivation and promote learning ability [1]. However, in contemporary society, particularly among individuals engaged in intellectual pursuits within universities or corporate settings, the frequent occurrence and chronic accumulation of anxious states have begun to impede cognitive function and even compromise physical well-being. For those enduring prolonged periods of anxiety without a clinical diagnosis of anxiety disorder, finding practical and effective methods to alleviate anxiety becomes a bewildering task, potentially exacerbating the emotional burden. Some may turn directly to medical intervention for assistance, yet some anti-anxiety medications based on neurotransmitters like serotonin and glutamate carry the potential for drug dependence and drowsiness [2,3]. On the other hand, alternative approaches to anxiety relief such as exercise prove challenging to implement for high-stress, fast-paced individuals.

Hence, in recent years, there has been growing interest in the study of natural compounds possessing anxiolytic properties. In aromatherapy, many essential oils, when administered through olfactory exposure, exhibit a marked capacity to alleviate stress-related emotions [4–6]. This allows this therapy to improve mood without directly oral taking or injecting drugs, making it widely applicable. Unlike drugs, essential oils are typically composed of hundreds of chemical constituents. The main components of many essential oils with anti-anxiety functions have also been proven to have certain anti-anxiety properties, and there may also be synergistic and antagonistic effects between compounds [7]. While it is highly likely that a predominant component exerts the primary effect, drawing definitive conclusions from the scent intervention of a single essential oil is insufficient. It is important to find key and effective compounds and clarify their modes of action, which is very helpful when judging the function of new essential oils or understanding the possible mechanisms of action of essential oils' function.

Linalool is widely found in many essential oils extracted from different parts of plants, like *Ocimum sanctum, Thymus vulgaris, Lavandula angustifolia,* and *Cananga odorata* [8]. Some previous studies have suggested that inhaling linalool has been shown to possess anxiolytic effects in mice or relaxing effects in human subjects [9,10], which were consistent with the effects observed in inhaling lavender [6,11] or bergamot essential oils [12], of whose shared linalool as the main components, as well as complex blends containing linalool [13,14]. Citral, a monoterpene with a lemon odor, is abundant in plants such as lemon, lemon balm, lemongrass, and *Lippia alba*. And the aroma of such plants is often confirmed to have a relieving effect on negative emotions in aromatherapy [15,16]. However, there are currently few reports on the use of citral to alleviate anxiety through olfactory pathways. β-Caryophyllene constitutes over 50 % of Copaiba essential oil [17] and is also found in other essential oils, such as *Spiranthera odoratissima* oil [18], *Artabotrys chitkokoi* oil [19], and *Caryophyllus aromaticus* oil [20]. In previous research, β-caryophyllene was found to interact with the CB_2 receptor, becoming the first known "dietary cannabinoid" with reported anxiolytic effects in numerous animal experiments [18,21,22], as well as antidepressant properties [18,23]. It can be inferred that β-caryophyllene likely possesses anxiolytic properties, yet empirical reports on its inhalation by humans to alleviate anxiety states are still lacking.

In traditional aromatherapy, it is believed that different types of essential oils may improve emotions in different ways, like increasing positive mood or making people more calm and relaxed. Previous research has found that the anti-anxiety mechanism of different types of essential oils or the main components of essential oils may be different. For instance, many studies have shown that the effects of essential oils are related to neurotransmitters. The anxiolytic effect of lavender essential oil was closely related to the serotonin system. Serotonin receptor antagonist WAY-100635, but not γ-aminobutyric acid A ($GABA_A$) receptor antagonist flumazenil blocked its anxiolytic effect [24]. A recent study indicated that the mechanisms involved in the action of different components in lavender essential oil are different, and related to the GABAergic system, cholinergic system, histaminergic system, and monoamines in the limbic system [25]. The anti-anxiety effect of some essential oils was also believed to be related to the GABA system. One study on lemongrass essential oil showed that the $GABA_A$ receptor antagonist picrotoxin could block its anxiolytic effect while WAY-100635 did not work [26]. The above research is mainly based on animal level, but it can be speculated that the mood regulation ways of inhaling different types of essential oils in people are also different, and different compounds may induce different physiological responses.

In light of these considerations, this study aims to explore the anxiolytic potential of β-caryophyllene, linalool, and citral inhalation under cognitive load, and discuss the psychological and physiological responses differences of the human body to the three compounds. The results may help to understand the differences in the mood regulation ways of essential oils with different odor types in aromatherapy. The cognitive tasks employed in this study include n-back tasks [27,28] and mental arithmetic exercises, both widely acknowledged for effectively inducing cognitive load and ensuing anxiety. To assess the impact of these tasks, we utilized a comprehensive set of multidimensional physiological and behavioral indicators to evaluate anxiety levels. These metrics encompass blood pressure [29,30], heart rate [29,30], blood oxygen saturation [17,31], fingertip temperature [32,33], facial expressions [33–35], and electroencephalogram (EEG) [36–38] measurements, all of which are associated with anxiety.

2. Materials and methods

2.1. Subjects

All the subjects were recruited through online questionnaires. This experiment required subjects to be 18–40 years old with no nasal diseases, no medical conditions that affect the sense ability of smell, no history of allergy to essential oils, and not under psychotropic treatment in the last three months. To avoid any potential negative odor impacts, females who were pregnant or at the stage of pregnancy preparation were excluded from the recruitment. Self-Rating Anxiety Scale (SAS) [39] was taken as the criteria for evaluating the anxiety level of candidates. A detailed description of the SAS can be found in the supplementary file. People whose raw score of SAS was greater than or equal to 36, which implied an anxious state, were recruited into this experiment. The sample size was

calculated using GPower software, and repeated measures were considered, with a two-sided 5 % significance level, 0.25 effect size, and a power of 80 %. A sample size of 10 subjects per group was necessary. 48 subjects (23 males and 25 females) were recruited and were stratified and randomly assigned to four intervention groups: β-caryophyllene group, linalool group, citral group, and the control group. All the subjects signed the informed consent forms. The study was approved by the Research and Ethics Offices of the Shanghai Jiao Tong University (No. H2021148I).

2.2. Odorants and odor environment

β-Caryophyllene (CAS: 87-44-5), linalool (CAS: 78-70-6), and citral (CAS: 5392-40-5), which were provided by Sinopharm chemical reagent Co., Ltd., were used as odorants in this experiment. Jojoba oil, used as the dilution of compounds and the odorant of the control group, was provided by the Aromatic Plant R&D Center of Shanghai Jiao Tong University. β-Caryophyllene, linalool, and citral were diluted using jojoba oil at a ratio of 1:1 by volume.

A specific space (1.70 × 1.70 × 1.85 m) was constructed as the test space using an opaque smooth plastic film cloth to control the test concentration while avoiding other external influences on the subjects. The temperature of the test space was maintained at 25 ± 2 °C. A table and a chair were placed in the space which allowed subjects to complete questions through the computer screen. When entering the odor intervention process, an aromatherapy incense diffuser placed in the corner was activated to atomize β-caryophyllene/linalool/citral/jojoba into the air. All personnel remained quiet during the test.

2.3. Determination of odor concentration in the test space

The odor concentration in the test space was measured and analyzed. The incense diffuser was turned on to release the odorants used in the experiment. An air sampling pump was used to extract 1 L of ambient gas inside the test space at 200 mL/min into the Tenax TA tube at 5, 10, and 15 min, respectively. A thermal desorption-gas chromatography (TD-GC) was used to analyze the chromatographic peak areas of β-caryophyllene, linalool, and citral at different time points. An external standard method was used for the quantitative determination of the compounds. The β-caryophyllene, linalool, and citral were diluted with methanol into 125, 250, 500, and 1000 ppm solutions, and 1.0 μL of each solution was injected into a Tenax TA tube. The chromatographic peak areas at different concentrations were detected using TD-GC. The standard curves can be graphed by linear fitting to calculate the odor concentration in the test space.

2.4. Mental workload task: n-back and mental arithmetic

N-back tasks and mental arithmetic tasks were used to impose an additional mental load on the subjects. Each task was conducted for 5 min at both training and test sessions. The n-back task requires subjects to observe a series of quick-presented stimulation and determine in each trial if the currently presented stimuli are the same as those presented in one or more ($n \geq 1$) trials before. In this study, 1-back tasks were shown through an online website (https://brainscale.net/dual-n-back). Nine small squares of equal area formed a large square area on the screen. Every 3 s, a visual stimulus accompanied by an auditory stimulus at the same time was presented for 500 ms in one of the squares randomly. The subjects were required to determine if the presented stimuli had any element (position, color, or audio) the same as the last one. The mental arithmetic tasks were also held through an online website (https://brainscale.net/mental-math), containing 30 trials of 3-digit addition with a time limit of 10 s per trial. In all tasks, the subjects were immediately notified when they had filled in an incorrect answer.

2.5. Self-report scales

2.5.1. The State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) was used as a scale of subjective self-assessment of anxiety level to compare the difference before and after odor intervention [40]. It consists of 2 subscales with a total of 40 descriptive questions: (1) State anxiety scale (S-AI). It describes an immediate or recent experience during a specific time and can be used to evaluate anxiety levels in stressful situations, with 20 questions included. (2) Trait anxiety scale (T-AI). It describes a relatively stable anxiety level that people experience usually for a long-term perspective. A detailed description of the STAI can be found in the supplementary file.

2.5.2. Subjective perception of environmental odors

The visual analogue scale (VAS) consisting of four dimensions (comfort, familiarity, pleasantness, and intensity) was used to assess the subjective perception of environmental odors. For comfort, familiarity, or pleasantness, a score of "-5" to "5" was assigned to the feeling of "very uncomfortable" to "very comfortable", "very unfamiliar" to "very unfamiliar", or "very unpleasant" to "very pleasant". A score from 1 to 6 was used to express the subjective intensity evaluation, representing "no smell", "almost unrecognizable smell", "slightly recognizable smell", "easily recognizable smell," "strong smell", and "very strong smell". A detailed description of the questionnaire can be found in the supplementary file.

2.6. Behavior measurements: facial expression

FaceReader (Noldus Information Technology bv), the software which was based on the Facial Action Coding System, FACS, was

used to recognize the subjects' facial expressions. Seven basic expressions, including neutral, happiness, sadness, anger, surprise, fear, and disgust, can be detected and quantified. The effectiveness East Asian model in recognizing and analyzing the facial expressions of Chinese people was verified to reach 71 %.

2.7. Physiological measurements

Heart rate (HR), blood pressure (BP), blood oxygen saturation (SpO_2), finger temperature (FT), and electroencephalogram (EEG) were five physiological indices to detect the emotional differences in subjects during the experiment. HR and BP including systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a digital blood pressure monitor (Omron). SpO_2 was measured by a finger clip oxygen saturation tester (Heal Force). The FT was measured by a dermatological instrument Tewameter TM210 (Courage + Khazaka Electronic GmbH).

The EEG was recorded by the Neuroscan Curry 8 system (Compumedics Limited). A cap (waveguard) was positioned on the subject's head according to the standard 10–20 system. EEG signals were collected from electrode sites in the frontal area (FP1, FP2, F3, F4, F7, F8, FZ), parietal area (C3, C4, CZ, P3, P4, PZ), temporal area (T7, T8), occipital area (O1, O2, P7, P8) at a sampling rate of 500 Hz, using FCz as the reference electrode. The electrode gel was used to make sure impedances were below 10K ohms. MATLAB R2020a and EEGLAB were used to analyze EEG data which were digitally filtered offline to a 1–30 Hz bandwidth. The power spectral density (PSD) of four spectra including delta (0.5–4 Hz), theta (4–7 Hz), alpha (8–12 Hz), and beta (13–30 Hz) bands was analyzed.

2.8. Procedure

Subjects were asked to avoid alcohol, nicotine, coffee, drugs, and the use of any scented products on the day of the test, and were not allowed to consume food for 1.5 h before the test. The experimental procedure (Fig. 1) was conducted in the following order: (1) Preparation. After being fitted with EEG equipment, the subjects were asked to sit in a comfortable position to rest for 10 min (2) Training session. The subjects were given a 5-min training session of the n-back task (n = 1) and a time-limited mental arithmetic task. (3) Testing session. The odor of β-caryophyllene/linalool/citral/jojoba started to release into the test space through an incense diffuser without informing the subject. The odor would be released for 20 min in total with a 5 s stop between every 60 s. Five minutes after the start of odor release, subjects performed the n-back task (n = 1) and the time-limited mental arithmetic task. The physiological parameters measurements including HR, BP, blood oxygen saturation, and finger temperature, were recorded before (T0) and afterthe training session (T1) and the test session (T2). Four segments of 5-min EEG (R1-R4) were recorded before and after the training session and test session separately. Facial expressions (S1-S4) were recorded before and during the training session and test session separately. STAI scale was filled by subjects after the training session and test session. After all the steps, an environmental odor evaluation scale was filled out as well.

2.9. Statistical analyses

One-way ANOVA and Tukey's multiple comparisons test were used to compare the difference of subjective environmental odor perception among four groups. Two-way ANOVA and Bonferroni's multiple comparisons were used to analyze the STAI, facial expression, and physiological measurements differences among four groups at different time points. One-way ANOVA and Bonferroni's multiple comparisons were used to analyze the variety rate of EEG among four groups.

3. Results

3.1. Impact of the odors on psychological indicators

The score differences in STAI before and after test sessions were illustrated in Fig. 2. Compared with the scores after the training session (T1), the scores of four groups all decreased in STAI ($F(1, 44) = 33.37, p < 0.01$), S-AI ($F(1, 44) = 26.53, p < 0.01$), and T-AI ($F(1, 44) = 14.77, p < 0.01$) after the test session (T2). Notably, only the β-caryophyllene intervention reduced the anxiety level significantly in STAI (Fig. 2A, $p < 0.001$) and the two sub-scales (Fig. 2B, $p < 0.01$; Fig. 2C, $p < 0.05$). The citral intervention reduced

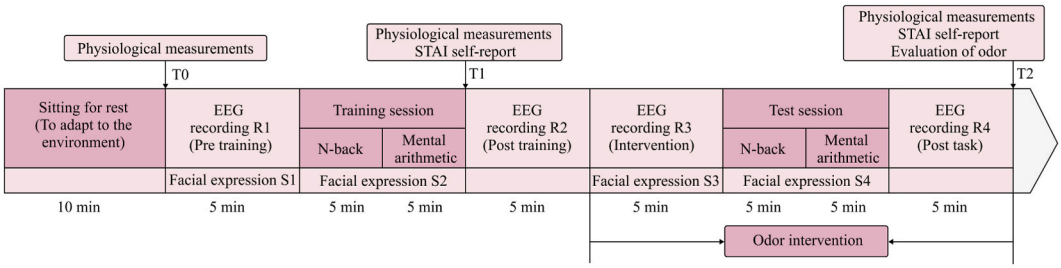

Fig. 1. Experiment procedure.

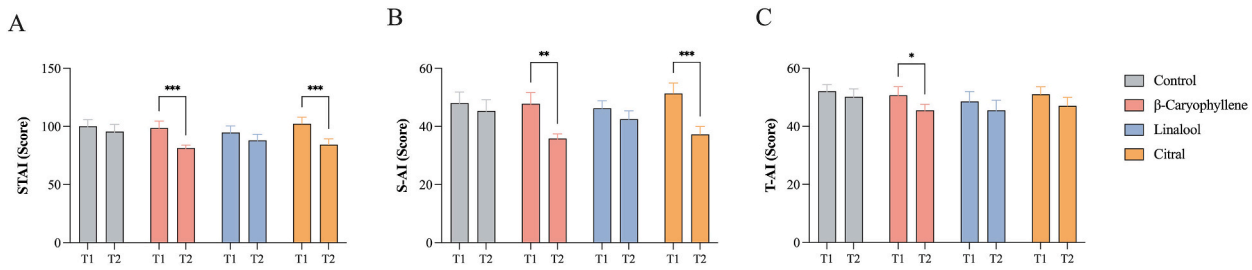

Fig. 2. STAI and the sub-scales before and after test sessions. Differences of STAI (A), S-AI (B) and T-AI (C) during T1 and T2. T1: time after the training session; T2: time after the test session. Two-way repeated ANOVA and Bonferroni's multiple comparisons test were used to analyze the difference among four groups before and after test sessions. Data was shown as mean ± SEM (n = 12). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

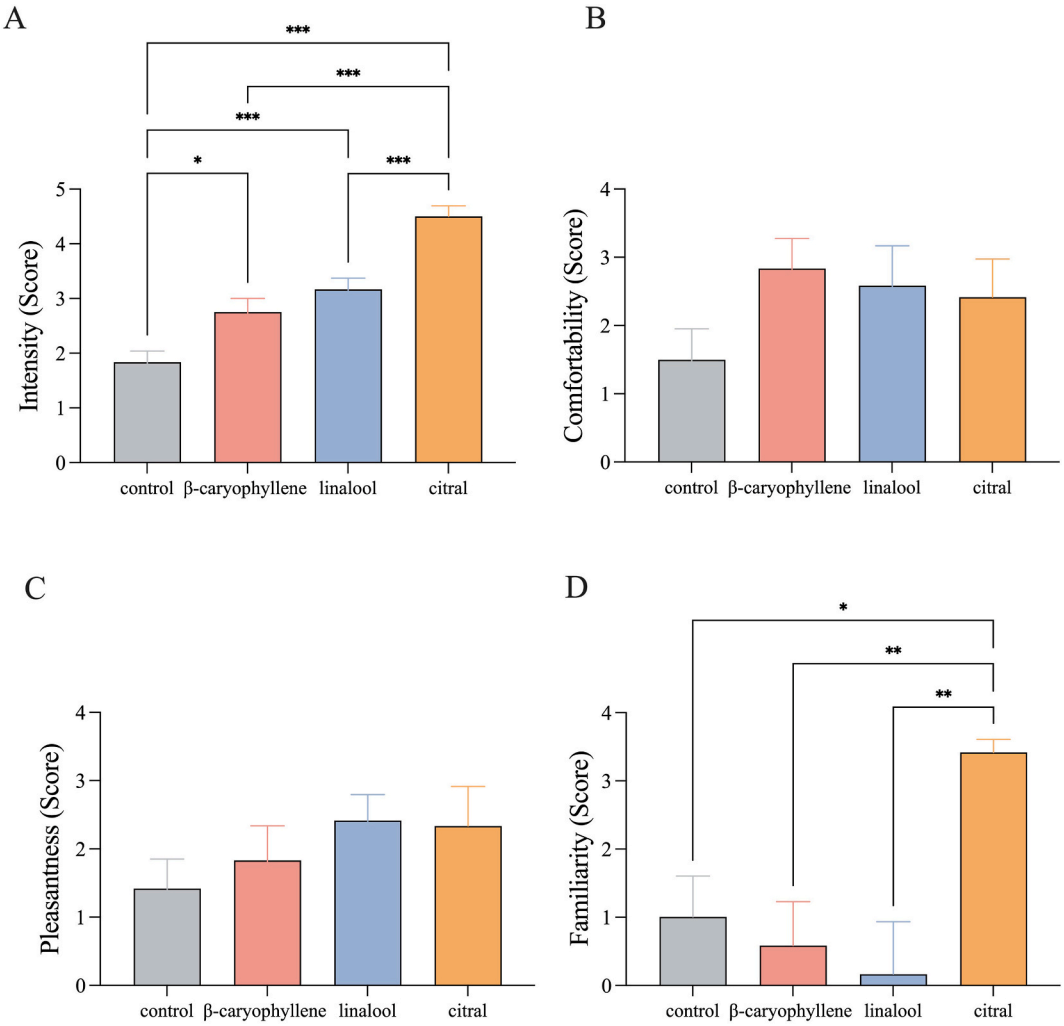

Fig. 3. The subjective assessment of environment odor perception. A. Intensity subjective assessment. B. Comfortability subjective assessment. C. Pleasantness subjective assessment. D. Familiarity subjective assessment. One-way ANOVA and Tukey's multiple comparisons test were used. Data was shown as mean ± SEM (n = 12). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the score of S-AI significantly (Fig. 2B, $p < 0.001$). In STAI and the sub-scales, scores at the same time point did not show any statistically significance between-group differences ($p > 0.05$).

3.2. Odor concentration and subjective perception of environmental odors

When compared to the control group, participants in the compound intervention groups reported a significant increase in odor intensity (Fig. 3A, $F(3, 44) = 26.39$, $p < 0.001$) among which citral showed the highest subjective intensity. In Fig. 3B, the comfort scores for β-caryophyllene, linalool, and citral groups were higher than those of the control group but with no statistical significance ($F(3, 44) = 1.29$, $p > 0.05$). Additionally, the pleasantness scores for all three experimental groups surpassed those of the control group (Fig. 3C) but showed no statistical significance ($F(3, 44) = 0.94$, $p > 0.05$). The familiarity showed a significant difference among the four groups (Fig. 3D, $F(3, 44) = 6.04$, $p < 0.01$). Scores of the citral group significantly differed from those of the other two groups and the control group ($p < 0.05$). The concentration of target compounds in the intervention environment during the 5–10, 10–15, and 15–20 min of intervention were analyzed. The results indicated that the concentrations of the three compounds were relatively stable during different periods of the environment (Table 1). At 15–20 min, the concentrations of β-caryophyllene, linalool, and citral in the environment were 24.8, 73.5, and 36.3 ppm, respectively.

3.3. Impact of the odors on physiology indicators

The SBP, DBP, HR, SpO_2, and FT at T0 (baseline measurements before all the tasks), T1 (measurements after the training session), and T2 (measurements after the test session) are shown in Fig. 4.

In Fig. 4A, the interactive effect of time point and odor intervention on SBP exhibited significantly ($F(3, 44) = 3.19$, $p < 0.05$). Compared with the baseline, the control group exhibited an increase trend in SBP after tasks, while all three odor treatment groups showed a decrease trend in SBP after the odor intervention. Only the linalool group demonstrated a significant decrease after the test session ($p < 0.05$). In Fig. 4B, the DBP of the three odor treatment groups also decreased, but without statistical significance ($F(3, 44) = 0.59$, $p > 0.05$).

As shown in Fig. 4C, there was a significant interactive effect of time point and odor intervention on HR ($F(6, 88) = 4.49$, $p < 0.001$). The control group exhibited a significant increase in HR compared to the baseline both after the training session ($p < 0.05$) and the test session ($p < 0.001$). After the test session with odor intervention, the HR of the β-caryophyllene ($p < 0.001$), linalool ($p < 0.001$), and citral group ($p < 0.05$) significantly decreased.

The SpO_2 of the β-caryophyllene group and the citral group decreased after odor intervention (Fig. 4D), although this change was not statistically significant ($F(6, 88) = 1.26$, $p > 0.05$). FT increased for both the β-caryophyllene group and the citral group (Fig. 4E), but without significance ($F(6, 88) = 0.47$, $p > 0.05$).

Across all five indicators, measurements at the same time point did not show any statistically significant between-group differences ($p > 0.05$).

3.4. Impact of the odors on facial expression

The facial expression data was analyzed at the baseline (S1), before (S3) and after (S4) the test session. Subjects received odor intervention continuously in the S3 and S4 stages. The results for the seven basic facial expressions during the three stages are presented in Fig. 5 (Netural, $F(2, 88) = 3.84$, $p < 0.05$; Happiness, $F(2, 88) = 2.10$, $p > 0.05$; Sadness, $F(2, 88) = 26.92$, $p < 0.001$; Anger, $F(2, 88) = 4.01$, $p < 0.05$; Surprise, $F(2, 88) = 6.06$, $p < 0.01$; Fear, $F(2, 88) = 4.32$, $p < 0.05$; Disgust, $F(2, 88) = 3.46$, $p < 0.05$).

In the control group (Fig. 5A), 'Sadness' ($p < 0.01$) and 'Fear' ($p < 0.05$) during S4 exhibited significant differences compared to S1. In the β-caryophyllene group (Fig. 5B), a significant decrease in 'Sadness' during S4 was observed compared to S1 ($p < 0.05$), while 'Happiness' during S4 increased significantly ($p < 0.05$). In the linalool group (Fig. 5C), 'Sadness' during S4 decreased significantly ($p < 0.001$) compared to S1. In the citral group (Fig. 5D), 'Neutral' during S4 increased significantly ($p < 0.05$) compared to S1.

3.5. Impact of the odors on EEG

The PSD variety rate of the four bands is shown in Fig. 6. R1 was a 5-min EEG recording before the training session, while R2 was that after the training session. R3 was a 5-min EEG but with odor intervention before the test session, while R4 was that after the test session with odor intervention. The PSD variety rate from R2 to R3 ((R3-R2)/R2), R2 to R4 ((R4-R2)/R2), and R3 to R4 ((R4-R3)/R3) were analyzed.

Table 1
Environmental odor concentration during the intervention.

Odorants	Concentration (ppb)		
	5–10 min	10–15 min	15–20 min
β-caryophyllene	18.1	23.8	24.8
linalool	68.3	86.3	73.5
citral	39.3	29.1	36.3

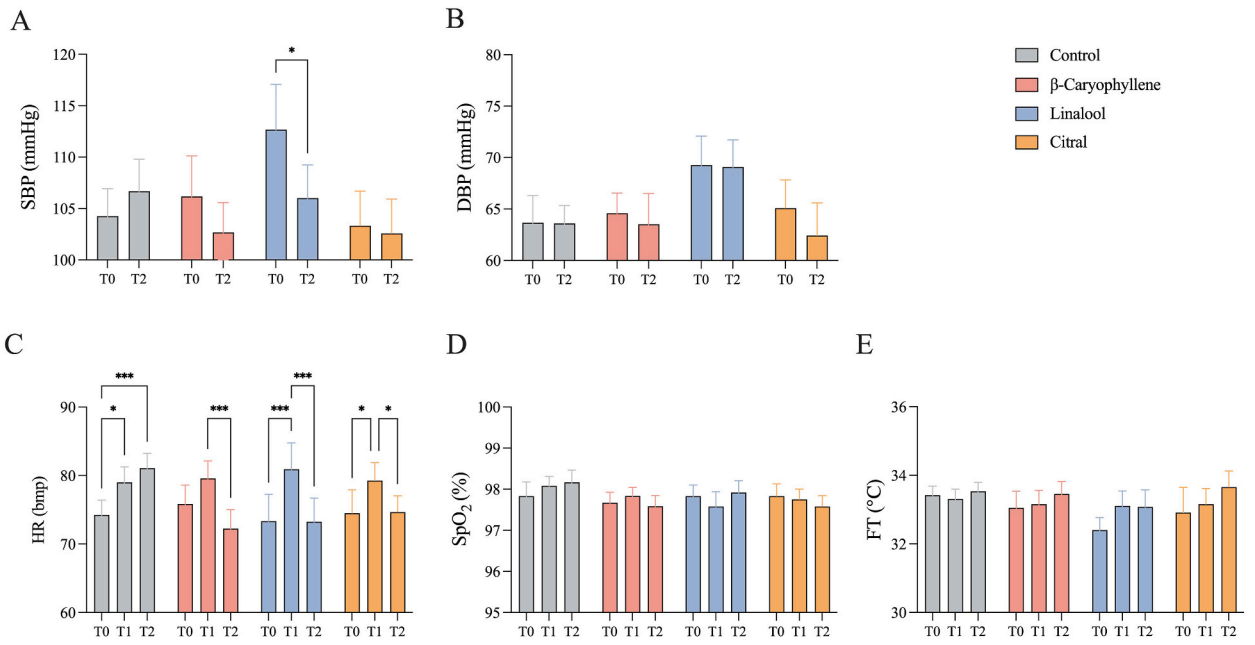

Fig. 4. Comparison of five physiological measurements at different time points. A. Systolic blood pressure (SBP) comparison before and after the whole experiment; B. Diastolic blood pressure (DBP) comparison before and after the whole experiment; C. Heart rate (HR) difference at three time points; D. Blood oxygen saturation (SpO_2) difference at three time points; E. Finger temperature (FT) difference at three time points. T0: baseline measurements before training session; T1: measurements after training session; T2: measurements after test session. Two-way repeated ANOVA and Bonferroni's multiple comparisons were used to analyze the differences among four groups at different time points. Data was shown as mean ± SEM (n = 12). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.5.1. EEG response change before and after the odor inhalation with no task (from R2 to R3)

The change rates from R2 to R3 of four bands in different brain regions were shown in Fig. 6A–D. It was used to analyze the anxiety-recovering effects of odor intervention.

Different intervention resulted in different EEG responses of alpha wave (occipital, F (3, 188) = 3.92, $p < 0.05$; parietal, F (3, 284) = 5.91, $p < 0.01$; frontal, F (3, 332) = 9.83, $p < 0.01$; temporal, F (3, 92) = 2.69, $p > 0.05$), beta wave (occipital, F (3,188) = 2.64, $p > 0.05$; parietal, F (3, 284) = 5.63, $p < 0.01$; frontal, F (3, 332) = 2,99, $p < 0.05$; temporal, F (3, 92) = 1.95, $p > 0.05$). In general, the β-caryophyllene and citral group mainly showed an apparent decreasing trend of four bands during the 5-min odor intervention. Compared with the control group, there was a significant reduction of the PSD of alpha wave in the frontal region of the citral ($p < 0.05$) and β-caryophyllene group ($p < 0.01$). The between-group of alpha wave comparisons (Fig. 6A) showed that the linalool group had a positive variety rate of PSD in parietal and frontal regions, which was significantly different from the citral($p < 0.01$) and β-caryophyllene group($p < 0.01$) in frontal and parietal regions.Only the linalool group had significantly elevated the PSD of beta wave compared with the control ($p < 0.01$) and β-caryophyllene ($p < 0.01$) group in the parietal region.

The differences of response of delta wave (occipital, F (3, 188) = 5.58, $p < 0.01$; parietal, F (3, 284) = 12.23, $p < 0.001$; frontal, F (3, 332) = 13.10, $p < 0.001$; temporal, F (3, 92) = 3.16, $p > 0.05$) and theta wave (occipital, F (3, 188) = 6.64, $p < 0.001$; parietal, F (3, 284) = 12.89, $p < 0.001$; frontal, F (3, 332) = 15.96, $p < 0.001$; temporal, F (3, 92) = 3.26, $p > 0.05$) were more specific. The linalool group showed an elevation of PSD of delta and theta waves in four regions, which had significant differences from the other two compounds intervention group (Fig. 6C and D). Compared with the control group, the PSD of the delta wave in the occipital, parietal, frontal, and temporal regions of the β-caryophyllene group significantly decreased ($p < 0.01$), while the PSD of theta wave in the occipital, parietal, frontal regions significantly decreased ($p < 0.05$). Moreover, the PSD of delta and theta waves in the frontal region of the citral group also significantly decreased ($p < 0.01$) compared with the control group.

3.5.2. EEG response change during odor inhalation before and after the task test (from R3 to R4)

The change rates from R3 to R4 of different regions of four bands are shown in Fig. 6E–H. It was used to analyze the anxiety-relieving effects of odor intervention during the brain-load tasks.

The different interventions resulted in different EEG responses of alpha and beta waves in occipital (alpha: F (3, 188) = 4.53, $p < 0.01$; beta: F (3, 188) = 3.10, $p < 0.05$) and frontal (alpha: F (3, 332) = 3.88, $p < 0.01$; beta: F (3, 332) = 4.57, $p < 0.01$) regions. The intervention has a more significant impact on the delta (occipital, F (3, 188) = 3.69, $p < 0.05$; parietal, F (3, 284) = 12.94, $p < 0.001$; frontal, F (3, 332) = 15.42, $p < 0.001$; temporal, F (3, 92) = 7.65, $p < 0.001$) and theta waves (occipital, F (3,188) = 2.74, $p < 0.05$; parietal, F (3, 284) = 8.89, $p < 0.001$; frontal, F (3, 332) = 13.41, $p < 0.001$; temporal, F (3, 92) = 7.02, $p < 0.001$) in different brain regions. Compared with the control group, only the PSD change of the beta wave of the β-caryophyllene group was significantly

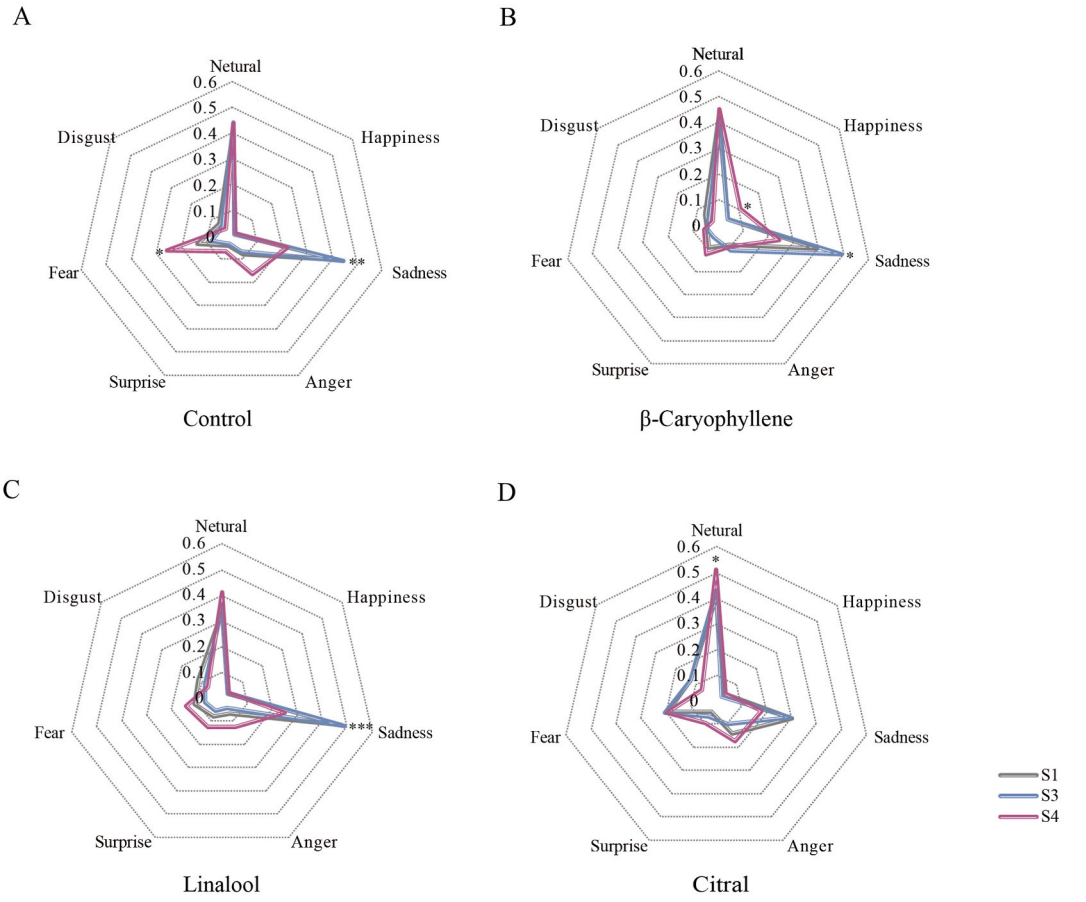

Fig. 5. Facial expression during different sessions. A. Facial expression differences in the control group; B. Facial expression differences in the β-caryophyllene group; C. Facial expression differences in the linalool group; D. Facial expression differences in the citral group. The values represent the proportion of different expressions recognized, with the seven basic expressions summing to 1. S1: 5-min baseline facial expression before the training session; S3: 5-min facial expression before the testing session; S4: 10-min facial expression data during the test session. Two-way repeated ANOVA and Bonferroni's multiple comparisons test was used to compare the facial difference between intervention sessions and the baseline. Data was shown as mean ± SEM (n = 12). The asterisk indicates that data in S4 is significantly different from S1. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

different in the occipital and frontal regions ($p < 0.05$). The elevation of PSD of alpha wave of the linalool and citral groups was more significant than the β-caryophyllene group ($p < 0.05$).

Compared with the control group, the PSD of delta and theta waves of all three compounds intervention groups showed a significant increase in parietal, frontal, and temporal regions ($p < 0.05$). Moreover, the PSD of the theta wave of the linalool group showed significantly increasing rates in the occipital region ($p < 0.05$), while the PSD of the delta wave of the β-caryophyllene and citral groups showed significantly increasing rates in the occipital region ($p < 0.05$). The enhancement amplitude of the delta wave in the parietal of the β-caryophyllene group was significantly higher than that in the citral and linalool groups ($p < 0.05$).

3.5.3. EEG response change before odor inhalation and after the task test (from R2 to R4)

The change rates from R2 to R4 of different regions of four bands are shown in Fig. 6I–L. It was used to comprehensively analyze the anxiety-relieving effects of odor intervention during the whole intervention session.

In general, different intervention resulted in different EEG responses of alpha, delta and theta waves in occipital (alpha: $F(3, 188) = 9.86$, $p < 0.001$; delta: $F(3, 188) = 5.41$, $p < 0.01$; theta: $F(3, 188) = 9.67$, $p < 0.001$), parietal (alpha: $F(3, 284) = 6.57$, $p < 0.001$; delta: $F(3, 284) = 6.40$, $p < 0.05$; theta: $F(3, 284) = 9.75$, $p < 0.001$) and frontal (alpha: $F(3, 332) = 15.60$, $p < 0.001$; delta: $F(3, 332) = 6.72$, $p < 0.001$; theta: $F(3, 332) = 9.91$, $p < 0.001$) regions. The intervention also affected the beta wave in the occipital ($F(3, 188) = 6.63$, $p < 0.001$) and frontal ($F(3, 332) = 11.20$, $p < 0.001$) regions.

Compared with the control group, the PSD of the alpha wave in the parietal region of the linalool group significantly raised ($p < 0.01$), while it decreased in the occipital and frontal regions of the β-caryophyllene group ($p < 0.05$). The PSD of the beta wave in the occipital and frontal regions of the β-caryophyllene group also significantly decreased ($p < 0.05$). For the PSD of theta and delta waves, only the linalool group showed a significant elevation compared to the control group in the occipital, parietal and frontal regions ($p <$

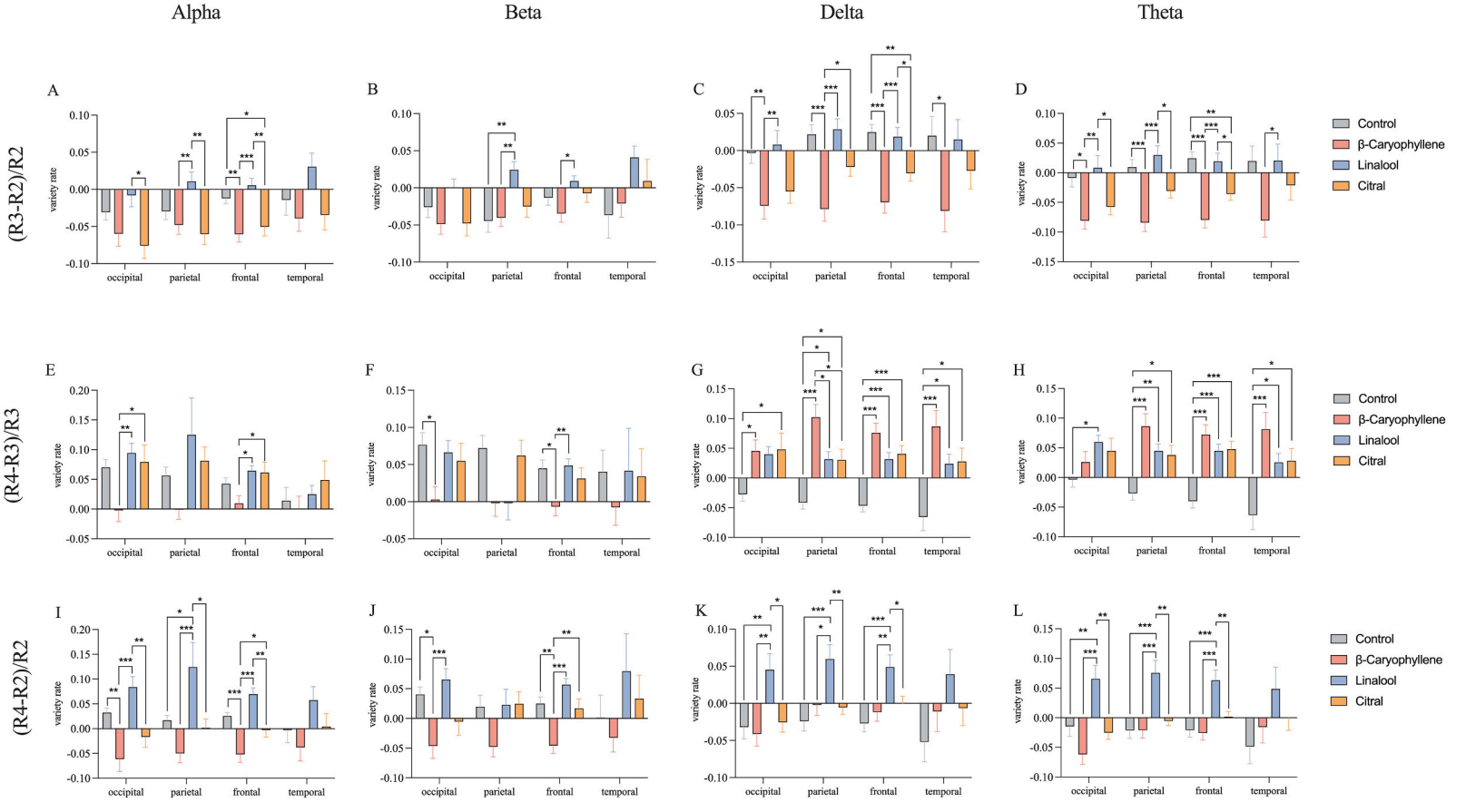

Fig. 6. Power spectral density (PSD) variety rate. A-D. Variety rate of PSD of alpha, beta, delta and theta waves from R2 to R3; E-H. Variety rate of PSD of alpha, beta, delta and theta waves from R3 to R4; I-L. Variety rate of PSD of alpha, beta, delta and theta waves from R2 to R4. R1: 5-min recording before the training session; R2: 5-min recording after the training session; R3: 5-min recording with odor intervention before the test session; R4: 5-min recording with odor intervention after the test session. All the variety rate calculation method was (pro-pre)/pre. One-way ANOVA and Bonferroni's multiple comparisons were used. Data was shown as mean ± SEM (n = 12). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

0.05).

4. Discussion

In aromatherapy, various essential oil aromas, such as the scents of lemon, lavender, and ylang-ylang oil, are believed to have anxiety-relieving effects. The chemical types of the primary compounds in these essential oils are different. Specifically, linalool is classified as a monoterpene alcohol, citral as a monoterpene aldehyde, and β-caryophyllene as a typical sesquiterpene. These compounds have been documented for their reported calming or anxiolytic properties. Research on citral and β-caryophyllene has primarily involved oral or injectable interventions, whereas olfactory exposure is a prevalent and safe method in aromatherapy. The present study aims to investigate the anxiety-alleviating effects of these three compounds through short-term olfactory exposure and to elucidate variations in anti-anxiety effects and physiological responses among them.

In this study, n-back and mental arithmetic tasks were used as stress stimulations to assess the effect of β-caryophyllene, linalool, and citral odors on anxiety-relieving. The subjects first experienced the training session of the n-back and mental arithmetic tasks and then received a continuous test session with odor intervention. Before and after these two sessions, the psychological and physiological indexes of the participants were recorded and analyzed.

The odor of β-caryophyllene demonstrated a noteworthy impact on anxiety relief, as evidenced by various subjective, physiological, and EEG measures within this study. Previous research [41–43] has utilized the STAI scale to evaluate the anxiety-relieving properties of essential oils. In this study, following the cognitive load task, both the β-caryophyllene and citral groups exhibited a significant reduction in STAI scores. However, only the β-caryophyllene intervention resulted in a significant decrease in both of its sub-scales (S-AI and T-AI). This indicateed that β-caryophyllene exhibits greater potential in alleviating acute anxiety. While numerous studies have demonstrated that the inhalation of linalool or lavender essential oils can reduce anxiety levels [44], our present study did not observe a significant STAI score decrease in the linalool group. A relevant study investigating the potential anxiety-reducing effects of lavender essential oil inhalation on preoperative anxiety in orthognathic surgery patients similarly reported that the lavender odor did not lead to a significant reduction in STAI scores [45]. Notably, the group exposed to a higher odor concentration exhibited a slightly lower STAI score. The observed performance of linalool on the STAI scale in the present study might be linked to the intervention concentration, which warranted further exploration.

Changes in HR and BP have been correlated with stress responses in individuals, with sympathetic activation in the autonomic nervous system often leading to an elevation of BP and HR. Previous studies have suggested that pleasant odors can have a calming effect on HR [46] and that exposure to essential oils like bitter orange and lavender, whether through inhalation or massage way, can lead to reduced heart rates [4,6]. Alterations in blood pressure have been observed following interventions with certain essential oils known for their sedative properties, such as ylang-ylang and bitter orange oils [29,30]. While all three odor interventions in the present study resulted in a decrease in blood pressure, only the linalool group exhibited a statistically significant change in SBP. This aligns with earlier findings indicating that linalool may possess some antihypertensive properties [47]. Furthermore, in contrast to the trend observed in the control group, all three odor interventions led to a reduction in heart rate after the cognitive task. The HR of the subjects was not affected by the control odor intervention and even showed a certain upward trend. This might be related to the cognitive tasks that the subjects took during the odor intervention phase.

Altered breathing patterns and rates can lead to reduced blood oxygen levels in situations of stress, anxiety, or excitement [48]. Besides, it has been proposed that anxiety-induced subcutaneous arterial vasoconstriction, aimed at decreasing peripheral blood flow, can result in lower fingertip temperatures and cold extremities [49]. However, in the present study, no significant changes in blood oxygen saturation or fingertip temperature were observed in any of the odor intervention groups.

Existing research has indicated an increase in alpha brainwave activity during relaxed states [50,51]. Beta wave activity usually decreases during drowsy states and increases with heightened alertness [52]. The increase in delta wave [53] and theta wave [54] suggests a state of relaxation. Several studies have shown that the enhancement of delta and theta waves is related to antidepressant and anti-anxiety behaviors [55,56]. In the present study, during the odor intervention in a non-task state (R3), compared with the control intervention, the citral odor significantly reduced the subjects' alpha wave, delta wave, and theta wave in the frontal region. The β-caryophyllene significantly reduced the alpha, delta, and theta waves. On the contrary, in the resting state after the n-back task (R4), the beta wave in the control group increased, while the delta and theta waves decreased. However, a significant increase in delta and theta waves was observed in the β-caryophyllene, linalool, and citral intervention groups. This suggested that in the non-task state, the first appearance of odor in the environment may enhance the subjects' alertness or cognition. However, after the subsequent task, subjects with three compounds odor intervention were more likely to calm down, especially the beta wave of the β-caryophyllene intervention group was lower than that of other groups in the frontal region, suggesting that subjects in the β-caryophyllene group might be less alert. When comparing the resting state EEG before and at the end of the intervention, it can be found that β-caryophyllene reduced the power of alpha and beta waves at the same time compared with the control intervention. Linalool also increased alpha, theta, and delta in three brain regions, which was consistent with previous studies [36,57].

In general, the responses in brainwave patterns between β-caryophyllene and citral are relatively close, while the distinctions from linalool are more significant, potentially tied to their respective mechanisms of action. Previous research suggested that the anxiolytic effects of β-caryophyllene involve the endocannabinoid system, as it can bind to CB_2 receptors, increase the expression of CB_2R proteins [58], thereby exerting neuroprotective effects, and improve neural damage [59]. The emotional regulation mechanisms associated with linalool are currently not conclusively determined. Some studies suggest its connection to the GABA system, proposing that the anxiolytic effects induced by linalool aroma may not involve serotonin transmission mediated by 5-HT1AR [60]. However, other research indicates that lavender essential oil, predominantly composed of linalool, achieves its anxiolytic effects through the

serotonin system rather than the GABA system [61]. It was found a significant increase in alpha waves in subjects after GABA intervention [62], aligning with the changes observed in the linalool group in this study, suggesting that the mechanism of action of linalool may be associated with the GABA system.

The results concerning facial expressions revealed a significant increase in the proportion score of 'Happiness' and a significant decrease in the proportion score of 'Fear' among subjects exposed to the β-caryophyllene odor intervention. This trend indicated a distinct anxiety-relieving focus for β-caryophyllene compared to the other two odors. When considering subjective perception ratings of the odor environment, it becomes evident that β-caryophyllene exhibited the lowest intensity, yet yielded the highest comfort score. While the comfort scores did not demonstrate statistical significance, this suggested that β-caryophyllene, despite its subtler presence, possesses equal soothing potential and may even enhance physiological pleasure. This characteristic may make it particularly well-suited for individuals sensitive to the intensity of odors.

However, this study possesses potential limitations. During the odor intervention, the intervention concentrations of the three compounds were not strictly consistent, though they were in an order of magnitude. As a result, the odor concentration was not strictly controlled. This issue was prevalent in numerous clinical studies of aromatherapy [63]. Employing a larger sample size and implementing an environment with precisely controlled odor concentrations would be beneficial for investigating potential variations in anxiolytic functions at different concentrations in future research. In addition, it is also meaningful to carry out further research to find the synergy between these compounds and related compounds.

5. Conclusions

In aromatherapy practice, various essential oils are recommended for anxiety relief. This study assessed the anxiety-relieving effects of β-caryophyllene, citral, and linalool in 48 subjects, utilizing n-back and mental arithmetic tasks as stress stimuli. The results demonstrated that the β-caryophyllene odor effectively alleviated anxiety, as evidenced by significant reductions in anxiety levels across both sub-scales of the STAI, a pronounced decline in heart rate, and a significant increase in the ratio of 'Happiness' facial expression coupled with a decrease in the ratio of "Fear" facial expression. Citral also exhibited noteworthy anti-anxiety effects, whereas linalool had a distinct impact on systolic blood pressure. EEG responses indicated unique emotional regulation pathways associated with the three compounds. In the non-task state, citral reduced frontal alpha, delta, and theta waves, with β-caryophyllene intervention producing a similar effect. Comparing EEG before and after the task, it can be found that all odor intervention groups displayed increased delta and theta waves compared to the control group, with the β-caryophyllene group notably exhibiting lower frontal beta waves. These findings offered insights into the potential differential effects of essential oil compounds. Larger sample size studies and controlled environments with adjustable odor concentrations are recommended for further exploration.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Shichun Pei: Writing – original draft, Investigation, Formal analysis. **Jie Chen:** Project administration, Investigation, Formal analysis. **Jing Lu:** Resources, Investigation. **Lei Yao:** Supervision, Funding acquisition, Conceptualization. **Nan Zhang:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38941.

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