

## The Effects of Beta-Ionone Addition in Perfume on Behavior and Serum Cortisol Level of Stress-Induced Mice

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### Abstract

Stress is a condition that triggers a change in behavior and physiological state, affecting mental health. Beta-Ionone is a monocyclic terpenoid compound that can lower stress levels. However, there is a lack of studies about the anti-stress capability of Beta-Ionone inhalation, although Beta-Ionone is commonly used as a fragrance in perfumes. This study aimed to evaluate the effect of Beta-Ionone addition as the fragrance in perfumes on mice stress. The Beta-Ionone perfume was formulated with Bergamot Oil, Lavender Oil, Eucalyptus Oil, Tea Tree Oil, and Patchouli Oil to produce Fem, Fem-Ion (Fem + Beta-Ionone), Masc, and Masc-Ion (Fem + Beta-Ionone), which had different preferable odors. The anti-stress effectivity was then evaluated in stress-induced mice, which were restrained for one hour every day for one week. The perfumes were then given by inhalation every alternate day. The stress level of the mice was evaluated using a tail suspension test and serum cortisol level assays. The results showed that inhalation of the perfumes lowered the immobility time of mice in the tail suspension test, albeit the addition of Beta-Ionone did not give a significant difference. Based on the results of serum cortisol level, there was a substantial decrease in serum cortisol by inhaling the Fem and Fem-Ion, showing decreases in stress level. However, there were increases in serum cortisol levels for Masc and Masc-Ion, indicating stress induction, which was suggested to be caused by 1,8-cineole, a compound in the Eucalyptus oil as the perfume oil components.

### Keywords

Beta-Ionone, Mental Health, Stress, Perfume, Serum Cortisol

Received: 8 November 2023, Accepted: 24 March 2024

<https://doi.org/10.26554/sti.2024.9.2.470-479>

## 1. INTRODUCTION

Stress is caused by uncontrolled physical demands, environment, and social situations (De Figueiredo et al., 2021). Through research conducted by Nochaiwong et al. (2021), it is estimated that 36.5% of the world's population experiences stress. Stress, especially in the long term, will cause behavioral changes in the form of decreased desire to socialize, sleep disorders, loss of drive to seek pleasure, and increased aggressiveness (Bombail, 2019; Lindsäter et al., 2021; Mariotti, 2015; Sandi and Haller, 2015).

Stress conditions cause activation of the hypothalamic-pituitary-adrenal axis (HPA axis), which plays a role in anxiety disorders and depression. Activation of the HPA axis stimulates the release of Corticotropin-releasing factor or CRF, which will then increase the release of cortisol (Kageyama et al., 2021). Cortisol is a glucocorticoid hormone that will increase fat and

glucose production breakdown so that the body has the energy needed by the heart, brain, and muscles in stressful conditions (Contrada and Baum, 2010; Thau L, 2024). Excess cortisol levels lead to anxiety disorders and depression and changes in behavior in the form of avoiding new situations or environments, shyness, and a tendency to be introverted (Dziurkowska and Wesolowski, 2021; Limone et al., 2021).

The secretion of cortisol by the adrenal glands begins with the amygdala activation by stress. The amygdala will then provide stimulation to the hypothalamus for CRF release to occur. Release from CRF will increase the pituitary's release of Adrenocorticotrophic hormone (ACTH) (Stephens and Wand, 2012; Kageyama et al., 2021). In the adrenal cortex, ACTH binds to receptors that cause cortisol secretion. ACTH and cortisol are always present in the blood in specific amounts. However, the ACTH hormone will be secreted excessively in severe stress conditions to produce optimal cortisol (Barret

et al., 2019). Excessive activation of the HPA axis caused by prolonged stress can cause structural changes in the HPA axis. These changes will lead to an increase in cortisol levels in the blood. This condition is often found in patients who suffer from depression, so it can be concluded that chronic stress can lead to depression (Contrada and Baum, 2010). Depression will cause difficulty in socializing and reduce productivity (Kupferberg and Hasler, 2023).

Based on research conducted by Park (2019), it is known that Beta-Ionone has anti-stress and antidepressant effects. Ketones in beta-Ionone can work as antagonists of CRF so that cortisol levels in the blood decrease. In research conducted on cells in human embryonic kidneys, HEK293 transfected with the CRF receptor gene reported that Beta-Ionone has effects as a CRF receptor antagonist. Meanwhile, in tests conducted on mice, it was found that Beta-Ionone can reduce stress and depression in mice. As an antagonist of the CRF receptor, Beta-Ionone will block the binding of CRF to its receptor so that the receptor is not activated. When CRF receptors are inactive, ACTH release will remain normal so that blood cortisol levels will remain normal (Kageyama et al., 2021). CRF receptors are known to occur in olfactory bulbs (Dedic et al., 2018). The olfactory bulb is located above the cribriform plate, thus separating the brain cavity from the top of the nasal cavity. The olfactory bulb is the part of brain tissue that grows anteriorly from the base of the brain. The olfactory bulb receives stimulation from olfactory cells in the nasal cavity and sends signals to other parts of the central nervous system (Binder et al., 2009; Hall and Hall, 2021).

Beta-Ionone is a volatile compound widely found in various plants, such as carrots, tomatoes, and apples. The smell of Beta-Ionone is described as violet (Paparella et al., 2021). Beta-Ionone has a boiling point of 271°C, a melting point of 35°C, a Log P value of 4.0, and a density of 0.940-0.947 g/cm<sup>3</sup>. It is water-soluble at 169 mg/L, soluble in alcohol, mostly fixed oil and propylene glycol, but insoluble in glycerin (National Center for Biotechnology Information, 2024). Beta-Ionone has been widely used in perfumes, soaps, cosmetics, and detergents (Lalko et al., 2007).

Fragrance selection in perfume will affect the sensory perception of its user. According to a previous study, the fragrance used affects the sensory perception of the product used topically, so the selection of fragrance is essential in improving the acceptability and quality of the product (Gonçalves et al., 2013). Moreover, scent will also affect a person's mood. From physiological observations, it can be known that a pleasant smell will cause a good mood, and a less pleasant smell will cause a bad mood (Kontaris et al., 2020). The appropriate fragrance selection will reduce stress and improve focus (Haehner et al., 2017).

At the present, no studies have explored the anti-stress effects of Beta-Ionone perfume. A previous study showed oral intake of Beta-Ionone-contained food at a dose of 4 mg/kg mouse body weight. It successfully improved the anti-stress behavior and reduced corticosterone plasma levels, indicating

anti-stress and anti-anxiety effects (Park, 2019). This study aims to formulate a Beta-Ionone perfume designed explicitly for its anti-stress properties. The use of perfumes will quickly deliver relaxing odorant molecules that can stimulate the reduction of stress while doing daily activities with no restrictions. It has also been known that perfume can affect the physiological mood to boost the mood and working capacity without difficulties (Sowndhararajan and Kim, 2016). Incorporating Beta-Ionone in the perfume is anticipated to facilitate inhalation and binding to CRF receptors in the olfactory bulbs.

Additionally, combining the formulation of Beta-Ionone perfume with five other essential oils boasting pleasant odors is expected to enhance further the anti-stress effects associated with Beta-Ionone use. The five essential oils used in this study include Bergamot, Eucalyptus, Lavender, Tea Tree, and Patchouli oils. Each of these essential oils is combined to achieve the desired pleasant smell of the perfumes, and they are also known to have stress-reducing, anti-anxiety, and vasorelaxation effects (Kim et al., 2014; Mannucci et al., 2017; Shin et al., 2020). Inhaling perfume is intended to achieve the anti-stress effect of Beta-Ionone with easy daily use.

In this study, the anti-stress effect of Beta-Ionone in perfume preparations was assessed by observing changes in mouse behavior through tail suspension tests and determining cortisol levels in serum. In stressful conditions, alterations in behavior and an elevation in serum cortisol levels are expected. Consequently, the evaluation test measured changes in mouse behavior returning to normal and decreased cortisol levels when Beta-Ionone was evaluated in this study.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

Beta-Ionone was purchased from Sigma-Aldrich Co.Ltd. (Saint Louis, USA) in this study. The essential oils, including Bergamot Oil, Lavender Oil, Tea Tree Oil, and Patchouli Oil, were bought from Jinshui Jinhai Natural Flavor Technology, Co.Ltd. (Ji'an, China). The Eucalyptus Oil was the product of Sima Arome (Pasuruan, Indonesia). The other components used in this study were Olive Oil (Filippo Berio, Massarosa, Italy), Sunflower Seed Oil (Moi Foods Malaysia, Selangor Darul Ehsan, Malaysia), Butylated Hydroxy Toluene (BHT) (Brat-aco, Bekasi, Indonesia), Benzophenone (Sigma-Aldrich, Saint Louis, USA), and Cortisol ELISA Kit (Ray Biotech, Norcross, USA).

### 2.2 Method

#### 2.2.1 Preparation of Beta-Ionone Perfumes

In this study, the perfumes were prepared according to the formula in Table 1. At first, BHT and benzophenone were dissolved in Olive Oil / Sunflower Seed Oil by stirring using a magnetic stirrer for 15 minutes at 1000 rpm. Then, fragrance base note, namely Patchouli Oil, was added and stirred until homogeneous mixtures were obtained. Then, the middle notes, including Beta-Ionone and Tea Tree Oil, were added, and the mixture was then stirred until homogeneous. In the last step,

the fragrance top notes, i.e., Lavender Oil, Bergamot Oil, and Eucalyptus Oil, were stirred until homogeneous. The perfume was then put into 5 mL vials with filler caps and allowed to stand at room temperature for 24 hours before being used in the test.

## 2.2.2 Characterization of Beta-Ionone Perfumes

### 2.2.2.1 Evaluation of Specific Gravity of Beta Ionone Perfume

The specific gravity test was conducted using a clean and dry pycnometer. Calibrated pycnometers were initially weighed when empty, then filled with boiling water, and then cooled to a temperature of 25°C. Following this, the temperature of the test substance was set to approximately 20°C, and the substance was added to the pycnometer until it reached total capacity. The temperature was then adjusted to 25°C. Any excess test substance was removed, and the pycnometer containing the substance was weighed. The weight of the substance or water was determined by subtracting the filled pycnometer's weight from the empty pycnometer's weight. The specific gravity of a substance was calculated by dividing the weight of the substance by the weight of water in the pycnometer (United States Pharmacopeia, 2023).

### 2.2.2.2 Qualitative Evaluation of Thin Layer Chromatography (TLC) of Beta Ionone Perfumes

The TLC tests were conducted to qualitatively analyze the profiles of oil components of beta-Ionone perfumes compared to the standard compounds. The TLC was performed on a Silica Gel plate 60 F254 with a toluene eluent: ethyl acetate (95 : 5) ratio. The sample was diluted with toluene before being applied to the TLC plate. After sample application, the plate was placed in a chamber containing saturated eluents. After development, the plate was allowed to dry and observed under UV lamps at 254 and 366 nm. Next, the plate was sprayed with an Anisaldehyde-Sulfuric Acid reagent composed of anisaldehyde: acetic acid:methanol: sulfuric acid (0.5 : 10 : 85 : 5) and heated on a hotplate at 105°C for 5 minutes until stains appeared.

## 2.2.3 In Vivo Anti-stress Efficacy Study of Beta-Ionone Perfumes

### 2.2.3.1 Experimental Animals

The ethical feasibility study for experimental animals was conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, on May 27, 2022, and ethical approval was obtained with the reference number 2.KEH.089.07.2022. This study involved male mice (*Mus musculus*) aged five weeks old. Before the testing, the mice had not been exposed to drugs or essential oils. Animals were randomly assigned to six groups, with the following group divisions: Group I (not stress-induced), Group II (stress-induced with no perfume), Group III (stress-induced and given Fem perfume formula), Group IV (stress-induced and given Fem-Ion perfume formula), Group V (stress-induced and given Masc perfume formula), and Group VI

(stress-induced and given the Masc-Ion perfume formula). The number of experimental animals used in this study was determined using Federer's formula:  $(n-1)(t-1) \geq 15$ , where  $n$  is the number of mice per group and  $t$  is the number of test groups. Based on these calculations, the number of mice per group was set at 4, resulting in 24 mice across the six groups.

### 2.2.3.2 Stress Induction to Mice

The group of mice that underwent behavior testing through the tail suspension test was subjected to stress induction by being confined in a 50 mL tube with ventilation holes at the ends, as illustrated in Figure 1a. Stress induction was conducted for one hour daily over 7 days (Schmidt et al., 2018).

### 2.2.3.3 Beta-Ionone Perfume Inhalation to Stress-induced Mice

The Beta-Ionone perfume was then administered by inhalation immediately after the completion of stress induction (Takemoto et al., 2020). The inhalation was carried out in acrylic cages measuring 15×10×10 cm, equipped with 1 cm diameter holes on the right and left sides, as depicted in Figure 1b. Before usage, each cage was cleaned with alcohol, wiped with 2% acetic acid, and allowed to dry. This procedure eliminated any lingering perfume scent from prior use. Subsequently, a cotton ball perfumed with 1 mL of perfume was positioned on the perforated plastic cup, which was then placed in the center of the cage and allowed to saturate for approximately 15 minutes before introducing the mice. Perfume inhalation by mice in acrylic cages is visually represented in Figure 1b. The inhalation was performed for 90 minutes on days 1, 3, 5, and 7 (Tsang et al., 2013).

### 2.2.3.4 In Vivo Evaluation of Stress Behaviour Using a Tail Suspension Test

Following perfume inhalation, the anti-stress behavior was evaluated using a tail suspension test. The mouse's tail was secured to the device, approximately 1/3 from the tip, ensuring the mouse's nose was positioned about 20 cm above the floor. The duration the mouse remained immobile with its head placed at the bottom, as illustrated in Figure 1c, was then measured. The test was conducted over 6 minutes (Wolffenbüttel et al., 2018).

### 2.2.3.5 Evaluation of Serum Cortisol Level in Stress-Induced Mice after Inhalation of Beta-Ionone Perfumes

The serum cortisol level test was conducted by extracting blood from mice undergoing perfume inhalation treatment. Mice selected for blood extraction were administered intraperitoneal ketamine anesthesia at 100 mg/kg body weight per mouse, and blood was collected through the inferior vena cava (Miatmoko et al., 2021). About 0.5 mL of blood was transferred into a pre-cooled centrifuge tube and centrifuged for 10 minutes at 1700xg force at 4°C to obtain (Gong et al., 2015). Subsequently, serum cortisol levels were quantified using the ELISA kit following the instructions (RayBiotech, Georgia, USA), and readings were taken at 450 nm.

**Table 1.** The Formulation of Beta-Ionone Perfumes

Components	Function	Concentration in the Formula (%)				
		Fem	Fem-ion	Masc	Masc-ion	
Lavender Oil	Fragrance (top notes)	1	1	2	2	
Bergamot Oil		0.4	0.4	0.4	0.4	
Eucalyptus Oil		0.3	0.3	2	2	
Beta-Ionone	Fragrance (middle notes)	-	4	-	4	
Tea Tree Oil		0.3	0.3	1	1	
Patchouli Oil	Fragrance (base notes)	8	8	10	10	
BHT		Antioxidant	0,2	0,2	0,2	0,2
Benzophenone		UV filter	0.5	0,5	0,5	0,5
Olive Oil	Solvent	89.3	85.3	-	-	
Sunflower Seed Oil	Solvent	-	-	83.9	79.9	

**Notes:**

Fem : The type I perfume formula with a memorable feminine odor

Fem-Ion : The type I perfume formula with a memorable feminine odor with the addition of Beta-Ionone

Masc : The type II perfume formula with a memorable masculine odor

Masc-Ion : The type II perfume formula has a memorable masculine odor with the addition of Beta-Ionone

**2.2.4 Data Analysis**

The data acquired in this study were analyzed utilizing the IBM SPSS computer program, version 25.0. The Independent T-test method was employed to identify significant differences among the perfume formulas created for weight data related to dosage types. For the *in vivo* studies, the one-way Analysis of Variance (ANOVA) method followed by the Tukey Honest Significant Difference post hoc test was applied to ascertain significant differences among the test groups.

**3. RESULTS AND DISCUSSIONS****3.1 Characterization of Beta-Ionone Perfume**

The parameter characterization of Beta-Ionone perfumes was conducted through a specific gravity test and a thin-layer chromatography (TLC) test.

**3.1.1 The Specific gravity of Beta-Ionone Perfumes**

The specific gravity of perfumes is presented in Figure 2a-b. The results showed that there were significant differences between Fem and Fem-Ion, as well as Masc and Masc-Ion. The addition of Beta-Ionone led to an increase in specific gravity in Fem and Masc perfumes.

The increase in specific gravity for Fem and Masc perfumes is attributed to the incorporation of 4% Beta-Ionone and the reduction of Olive Oil or Sunflower Seed Oil by a total of 4%, forming the solvent of the perfume formula. Olive Oil has a specific gravity of 0.908 g/cm<sup>3</sup>, Sunflower Seed Oil has a specific gravity of 0.9157, and Beta-Ionone ranges from 0.940-0.947 g/cm<sup>3</sup> (Ichu and Nwakanma, 2019; National Center for Biotechnology Information, 2024; Oguche, 2021). Given that Olive Oil and Sunflower Seed Oil have a lower

specific gravity than that of Beta-Ionone, the addition of Beta-Ionone results in an increased specific gravity for Fem and Masc perfumes. Furthermore, based on the specific gravity test results, all perfume formulas comply with the specific gravity requirements outlined in the Standar Nasional Indonesia (SNI) 16-4949-1998, namely, 0.7-1.2 g/cm<sup>3</sup> (Badan Standarisasi Nasional (National Standardization Agency), 1998)

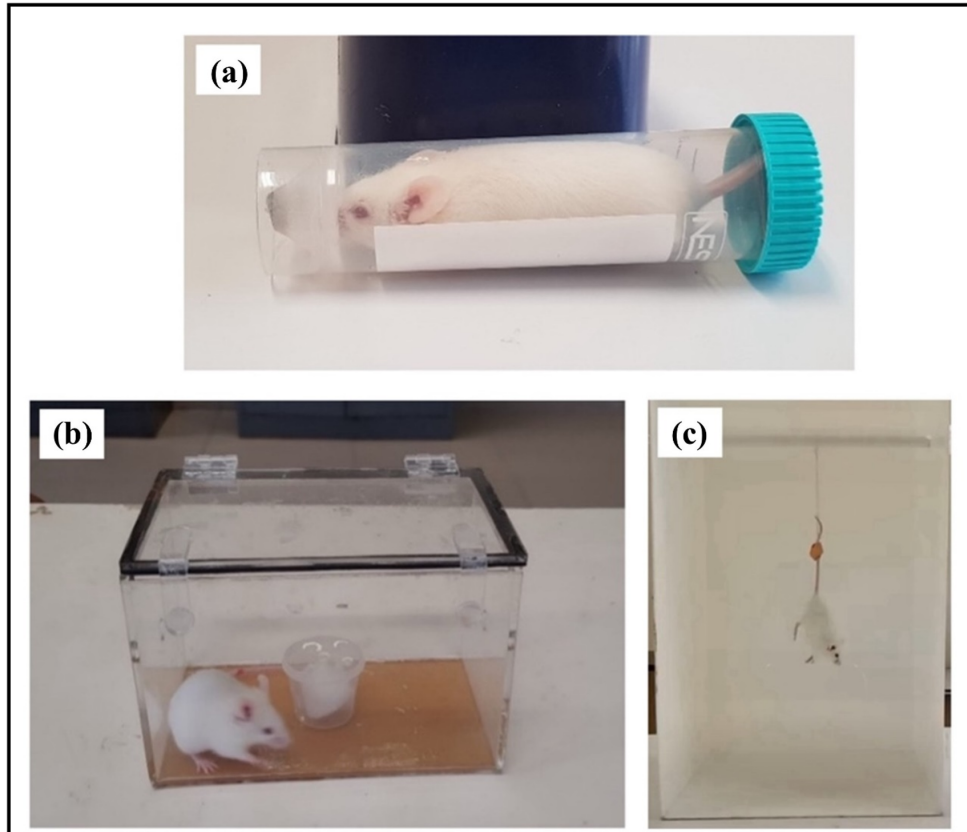
**3.1.2 Thin Layer Chromatography (TLC)**

Based on the results obtained from the Thin Layer Chromatography (TLC) analysis of Bergamot Oil (BGO), Eucalyptus Oil (EO), Lavender Oil (LO), Beta-Ionone (BIO), Tea Tree Oil (TTO), Patchouli Oil (PO), Fem Perfume (F), Fem-Ion Perfume (FI), Masc Perfume (M), and Masc-Ion Perfume (MI), the tested samples, namely Fem, Fem-Ion, Masc, and Masc-Ion perfumes, exhibited identical amounts, colors, and sizes of stains as shown in Figure 3. All stains observed on the samples align with those appearing on the standard; in other words, the oil components were still present in the perfumes.

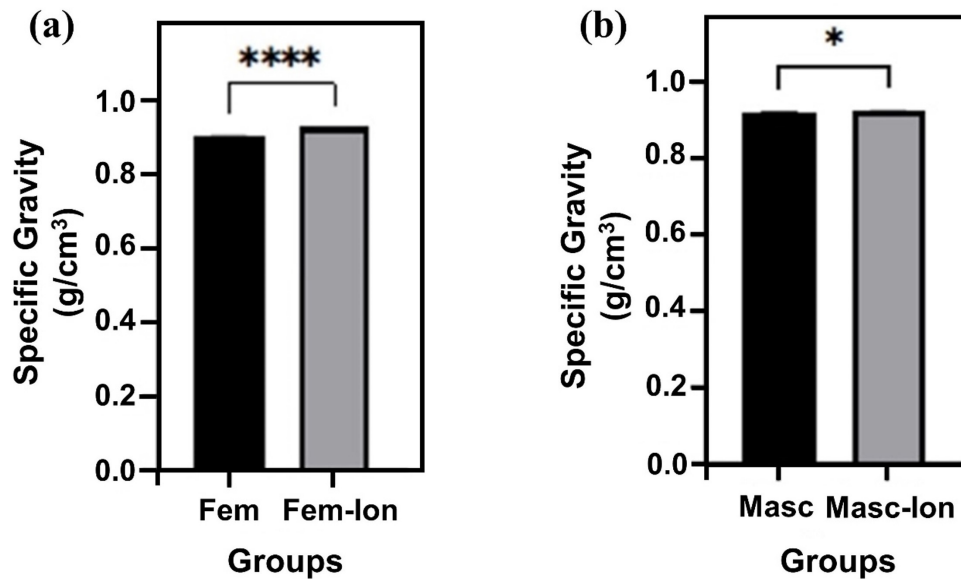
**3.2 In Vivo Anti-stress Efficacy Study of Beta-Ionone Perfumes****3.2.1 Optimization of Tail Suspension Test**

The average immobility time results of mice in the tail suspension test optimization were presented in Figure 4. The optimization process involved the use of three mice in each group. Stress induction was performed by confining mice in a 50 mL tube with ventilation holes for 14 consecutive days. The tail suspension tests were conducted on days 7 and 14.

Based on the optimization results of the tail suspension test, inducing stress for seven days significantly increased immobility time compared to normal mice. However, inducing



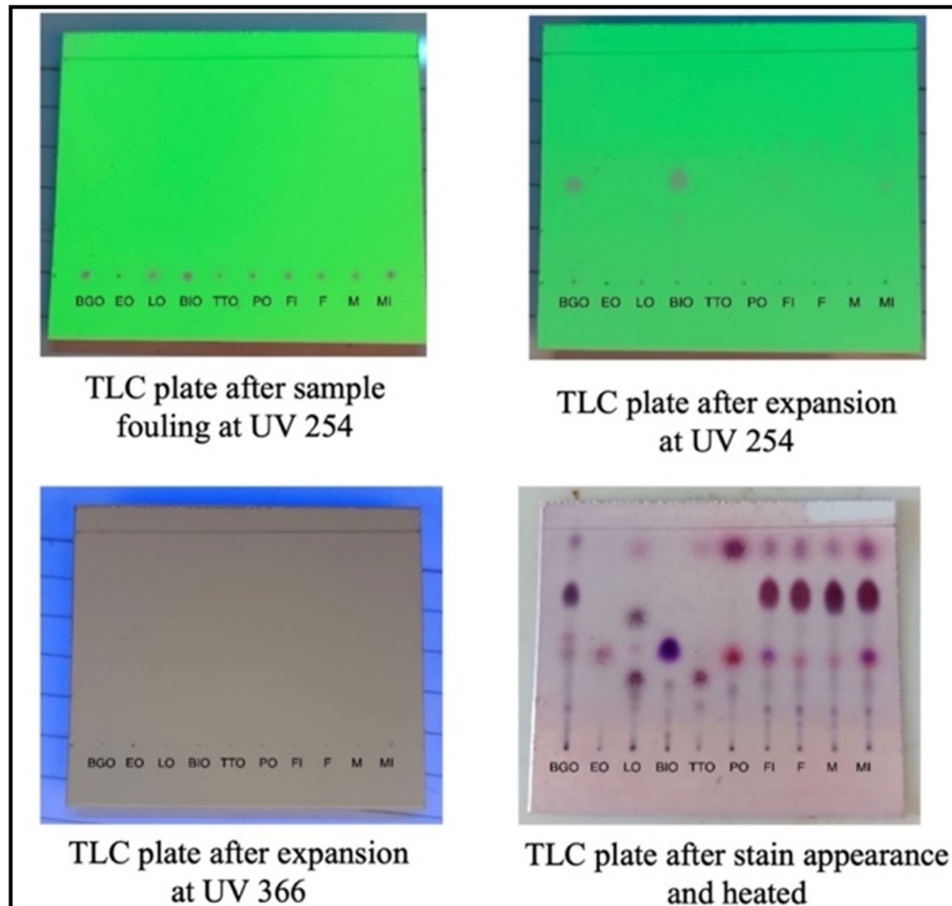
**Figure 1.** The Figures of Experimental Conditions: Stress Induction of Mice Using a Tube, (a) Inhalation of Perfume on Acrylic Cages for *In Vivo* Anti-Stress Efficacy Study (b), and Tail Suspension Test on Mouse (c)



**Figure 2.** The Specific Gravity of Feminine (a) and Masculine (b) Preferable Perfumes. \* $P \leq 0.05$  and \*\*\*\* $P \leq 0.0001$

stress for 14 days did not significantly differ from the normal group. Therefore, in this study, stress induction was conducted

for seven days. The decrease in immobility time observed after prolonged stress induction may be attributed to resilience,



**Figure 3.** Thin Layer Chromatograms of Perfumes Analyzed on TLC GF 254 Plate Size 5.5×5 cm with Toluene Eluent: Ethyl Acetate as the Mobile Phase and Anisaldehyd-Sulphuric Acid Staining Agents. BGO: Bergamot Oil, EO: Eucalyptus Oil, LO: Lavender Oil, BIO: Beta Ionone, TTO: Tea Tree Oil, PO: Patchouli Oil, F: Fem, FI: Fem Ion, M: Masc, MI: Masc Ion

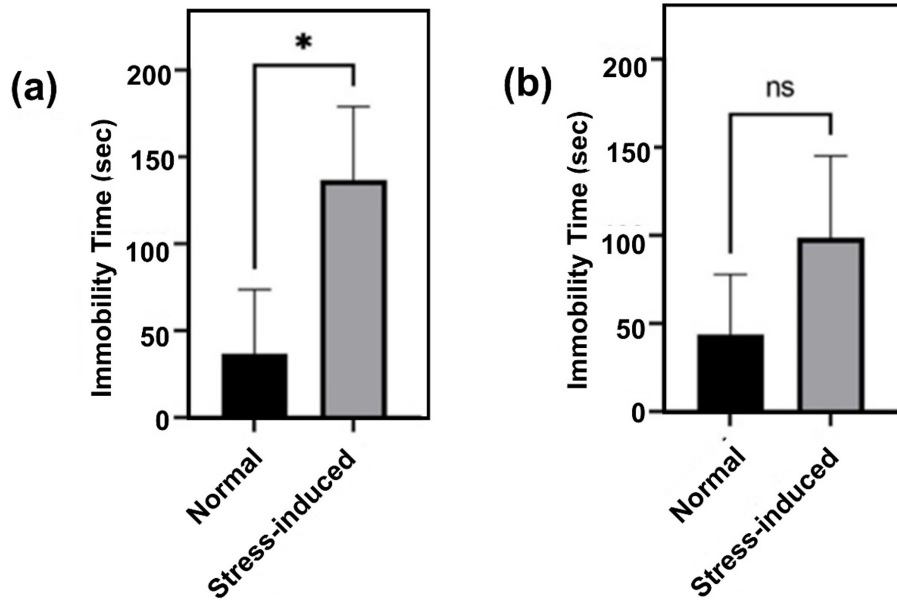
wherein mice adapt to conditions of repeated stress (Lyons et al., 2017).

The tail suspension test was optimized using mice of varying ages compared to those in the initial study. According to the research conducted by Shoji et al. (2016), mice's age variations resulted in a reduction in immobility time during the tail suspension test. Age differences can contribute to distinct responses to the applied stress. This variance may elucidate the disparities between the results obtained during the optimization phase and the subsequent research.

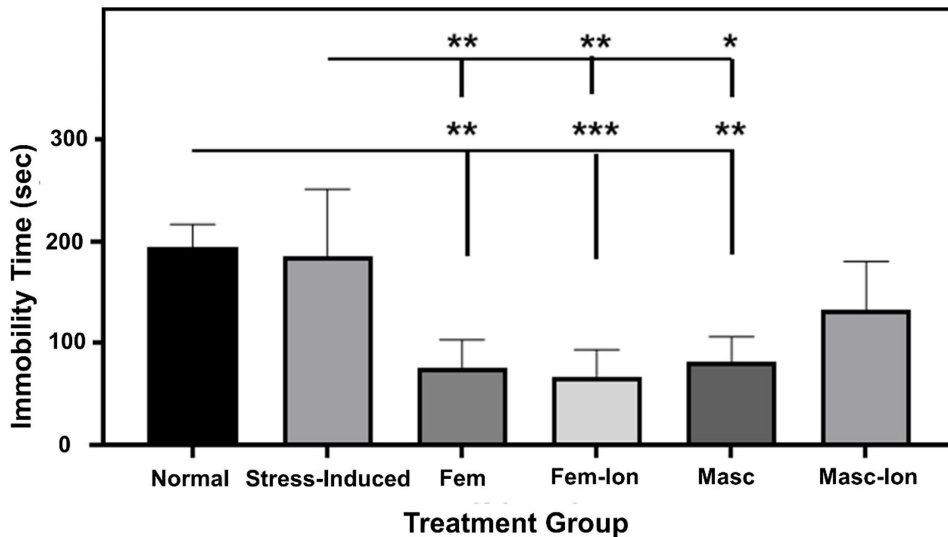
### 3.2.2 *In Vivo* Evaluation of Stress Behaviour Using a Tail Suspension Test

The results of the average immobility time of stress-induced mice after perfume inhalation evaluated by the tail suspension test can be observed in Figure 5. Based on the results, all mice exposed to perfume inhalation exhibited decreases in immobility time, indicating a reduction in depression-like behavior triggered by stressful conditions. This decrease in immobility time was observed in mice exposed to perfume

compared to normal and stress-induced mice without perfume. Through One-Way ANOVA analysis, significant differences were found in the immobility time among the Fem, Fem-Ion, and Masc treatment groups when compared to both normal and stress-induced groups. However, it is noteworthy that there was a decrease in immobility time observed in both normal and stress-induced mice. Previous study shows that chronic stress induction through restraint may decrease immobility time in the tail suspension test (Medina-Rodriguez et al., 2022). The hypothesis in this study posited that the reduction in immobility time in the stress group may be attributed to the heightened anxiety experienced by the mice. Chronic and acute stress can induce changes in the brain's regulatory activity of noreadrenaline, dopamine, and serotonin (Seki et al., 2018). In conditions of chronic restraint-induced stress, it is suspected that there is an increase in sporogenic activity in the brain, resulting in a decrease in immobility time.



**Figure 4.** The Graph of Mean Immobility Time of Normal and Stress-Induced Mice Evaluated with Tail Suspension Test Optimization After Stress Induction for 7 Days (a) and 14 Days (b). \* $P \leq 0.05$  and ns = Not Significant ( $P > 0.05$ )



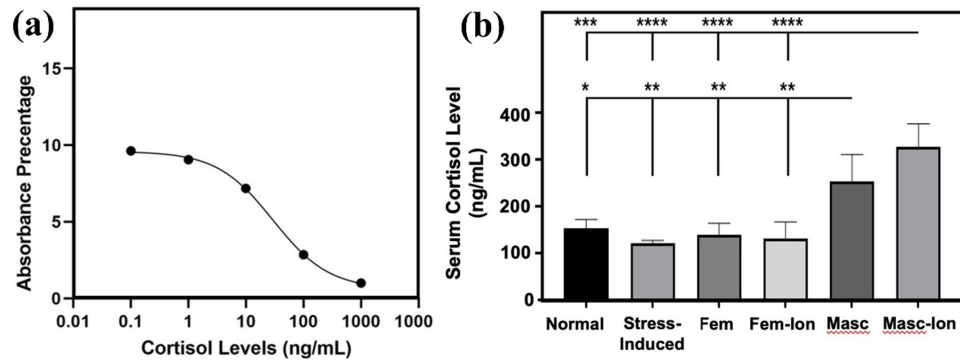
**Figure 5.** The Graph of Mean Immobility Time was Evaluated by a Tail Suspension Test After Perfume Inhalation on Stress-Induced Mice. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

**3.2.3 In Vivo Evaluation of Serum Cortisol Level**

After the tail suspension test, blood was drawn from the mice, and serum cortisol levels were measured using ELISA. The standard curve for cortisol levels can be observed in Figure 6a. After regression analysis, it was determined that the  $R_2$  of the standard curve was 0.9995. The regression equation derived from this analysis is utilized to ascertain serum cortisol levels in the samples.

Based on the results of serum cortisol levels as presented

in Figure 6b, no significant differences were observed among the normal, stress-induced, Fem, and Fem-Ion mice groups. However, significant reductions in serum cortisol levels were found in Fem and Fem-Ion groups when compared to the Masc and Masc-Ion groups. Considering the physicochemical characteristic of Beta-Ionone, theoretically, Beta-Ionone cannot penetrate the blood-brain barrier due to the optimal blood-brain barrier penetration in logP, which typically ranges between 1.5 and 2.7. At the same time, Beta-Ionone has a



**Figure 6.** Standard Curve of Cortisol Levels (a) and the Graphic of Mean Serum Cortisol Levels After Perfume Inhalation on Stress-Induced Mice (b). \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; and \*\*\*\* $P \leq 0.0001$

log P value of 4.0. The effects of Beta-Ionone on stress may be more related to receptors outside the blood-brain barrier. Overall, there were no significant differences observed for Beta-Ionone addition, which were between the Fem and Fem-Ion groups, as well as the Masc and Masc-Ion groups. These results are probably due to a lack of concentration of Beta-Ionone, which may need to be increased to enhance anti-stress efficacy significantly. Thus, further research on adding beta-Ionone in various concentrations is needed to determine the optimal concentration.

In this study, the Masc and Masc-Ion groups' immobility time was lower than the stress-induced group. However, the serum cortisol levels of both groups were higher, even compared to the normal group. According to a study by Rahman et al. (2013), inhaling Eucalyptus Oil can produce an anxiogenic effect in mice. In addition, 1,8 cineole has been suggested to enhance locomotor activity, which is related to increased alertness (Moss and Oliver, 2012). The 1,8-cineole compound is commonly found in Eucalyptus Oil and Tea Tree Oil (Borotová et al., 2022; Hoch et al., 2023). Therefore, it is probably due to mice exposed to perfume inhalation containing Eucalyptus Oil causing a significant increase in serum cortisol levels in the Masc and Masc-Ion groups, which contained larger amounts of Eucalyptus oil than those of Fem and Fem-Ion. This increase is suspected to result from the stimulation of olfactory organs and direct stimulation of the central nervous system (CNS) (Moss and Oliver, 2012). Therefore, the presence of 1,8-cineole in these oil components might affect the tail suspension test results and cortisol assay results. However, the exact mechanism of 1,8-cineole in the CNS is unknown. Moreover, multiple essential oils have been used to prepare Beta-Ionone perfumes that may cause synergistic, additive, or antagonistic effects of the anti-stress activity. Further evaluation of other parameters related to anti-stress efficacy is needed to propose using Beta-Ionone and its combination with other essential oils for producing perfumes.

#### 4. CONCLUSIONS

Perfume inhalation containing Bergamot Oil, Eucalyptus Oil, Lavender Oil, Tea Tree Oil, Patchouli Oil, and Beta-Ionone successfully reduced the immobility time of stress-induced mice. However, adding Beta-Ionone to the perfumes had no significant effect on anti-stress efficacy. Adding Beta-Ionone to perfume also had no significant effect on the serum cortisol levels of stress-induced mice. It is predicted that 1,8-cineole, a terpenoid compound presented in the Eucalyptus and Tea Tree oils, increased the stress in mice. Further comprehensive studies on related stress parameters in stress-induced mice are required to propose using Beta-Ionone and multiple essential oils as the perfume components for enhancing stress reduction.

#### 5. ACKNOWLEDGMENT

The authors thank Dr. Melanny Ika Sulistyawati and Ms. Rifda Tarimi Octavia for providing material support and kind assistantship in the manuscript writing of this study.

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