*Original Article*

# Exploring the Calming Potential of Peppermint: Anxiolytic Effects of *Mentha piperita* Essential Oil on State and Trait Anxiety

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**ABSTRACT**
**Introduction:** This study aimed to evaluate the anxiolytic effect of *Mentha piperita* essential oil on state and trait anxiety. **Methods:** The essential oil was extracted by hydrodistillation, and its chemical composition was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). A total of 93 participants were divided into a control group (CG) and an experimental group (EG), with 47 participants in the EG receiving MPEO aromatherapy and 46 in the CG. Anxiety levels were measured using the State-Trait Anxiety Inventory (STAI) at pretest and posttest phases. **Results:** GC-MS analysis showed that menthol had the highest concentration (42.56%), followed by menthone (19.24%) and linalool (11.68%). Mixed model analysis revealed that group assignment (EG vs. CG) was a significant predictor of posttest state anxiety (F=16.508, p=0.001) and trait anxiety (F=9.091, p=0.003), independent of pretest scores, supporting the intervention's effect on reducing anxiety. Posttest results showed a significant reduction in state anxiety in the EG compared to the CG (p=0.001) with a large effect size (r=0.508), while trait anxiety showed a moderate reduction (p=0.001, r=0.342). Conclusion: These findings suggest that *Mentha piperita* essential oil may effectively reduce state and trait anxiety, with a more pronounced impact on state anxiety.
**Keywords:** Mentha piperita, Essential oil, Anxiety, State anxiety, Trait anxiety.

## INTRODUCTION

Approximately 4.05% of the worldwide population, equivalent to 301 million individuals, are affected by an anxiety illness. The measurements for anxiety disorders indicate a consistent rise in the prevalence, incidence, and rates of disability-adjusted life years (DALYs) over the last three decades [1].

Anxiety disorders give rise to a range of negative outcomes, encompassing impaired social, occupational, and personal functioning, as well as physical and cognitive manifestations such as lethargy, fatigue, irritability, and concentration challenges[2]. Besides, these disorders have a substantial impact on mortality risk, particularly pronounced when anxiety disorders coexist with depression, which has significant implications for public health[3].

Conventional approaches for treating anxiety usually encompass a blend of pharmacotherapy and psychotherapeutic techniques[4]. Benzodiazepines (BDZs) and antidepressants are frequently used in pharmacological treatment for anxiety; however, BDZs cause adverse effects, including lethargy, retrograde amnesia, drowsiness, disorientation, and vertigo [5]. In addition, the literature indicates that psychotherapies, such as cognitive behavioral therapy, have long-term benefits for patients with anxiety problems but with high dropout rates [6–8].

In this regard, non-pharmaceutical therapy has drawn a lot of interest[9]. Studies in herbal therapy have shown that a range of therapeutic herbs may help treat anxiety[10,11], emphasizing essential oils' (EOs) use as a helpful substitute therapy for anxiety[12]. Essential oils, such as lavender, ylang-ylang, marjoram, and neroli, have decreased blood pressure, reduced cortisol levels, and promoted relaxation, which can help alleviate anxiety symptoms[13]. Aromatherapy has been found to have positive anxiolytic effects on individuals with anxiety symptoms, with no reported adverse effects because it is recommended as a complementary therapy for managing anxiety[14].

*Mentha piperita L. (peppermint),* a hybrid of *Mentha aquatica* (watermint) and *Mentha spicata* (spearmint), is one of the most widely used and common species worldwide. It is valued mainly for its medicinal, industrial, and culinary applications, with its significance primarily derived from its essential oils and polyphenols[15]. A study found that peppermint essential oil aromatherapy reduced anxiety in patients with acute coronary syndrome[16]. Additionally, research showed that peppermint aromatherapy alleviated discomfort and anxiety related to intravenous catheterization, recommending its use before the procedure[17]. Similarly, another investigation found that peppermint aromatherapy reduced anxiety during labor in nulliparous women, reinforcing its potential as a complementary approach in obstetric care [18]. In this study, we aimed to evaluate the anxiolytic effect of *Mentha piperita* essential oil on state and trait anxiety.

## MATERIAL AND METHODS

### Plant material

*Mentha piperita leaves* were purchased from a Peruvian supplier (Ayacucho, Perú). The samples were pulverized, and the powdered plant material (100g) was placed in a round-bottom flask containing 1000 ml of distilled water, which was then linked to

a modified Clevenger-type device. Hydrodistillation was conducted for three hours post-boiling. The oil was subsequently dried using anhydrous sodium sulfate (Na2SO4) and preserved in amber glass vials at +4°C in a refrigerator for future experimental applications.

## Determination of essential oil composition

The gas chromatography-mass spectrometry (GC/MS) studies were conducted on a Hewlett-Packard 6890 gas chromatograph coupled with an HP-5MS column (30 m x 0.25 mm inner diameter, 0.25 μm film thickness) and a Hewlett-Packard 5972 mass spectrometer. The column's initial temperature was set at 50 °C and maintained for 5 minutes. The temperature was then raised by 5 °C per minute until reaching 240 °C. The final step involved increasing the temperature by 15 °C per minute to 300 °C, where it was held for 2 minutes. The injector port temperature was set at 250 °C, and helium was used as the carrier gas at a 0.9 mL/min flow rate. The mass spectrometer operated in electron impact mode (EI) with an ionization voltage of 70 eV and an ion source temperature of 250 °C. Essential oil constituents were identified by comparing their mass spectra and retention indices (RI) with those from the NIST 2011 mass spectra library and the Wiley and Adams libraries[19,20].

## Study design and sample

An experimental study with measures at pretest-posttest was conducted. The sample size was estimated at 48 individuals per group using the mean difference formula for two independent populations. The calculation was based on a previous study[16] that reported a mean and standard deviation of patients' anxiety of 37.72 ± 10.41 in the intervention group and 42.62 ± 5.99 in the control group. The sample size calculation was conducted using a 95% confidence interval, 80% test power, and according to the two-tailed test. This yielded a required sample size of 48 individuals per group. Minor participant attrition resulted in 47 participants in the experimental group (EG) and 46 in the control group (CG) at the end of the study. These minimal losses did not substantially impact the overall power and validity of the investigation.

## Instruments

**State-Trait Anxiety Inventory (STAI):** It consists of two self-report scales measuring two distinct types of anxiety: state and trait. Both scales have 20 statements; respondents score their sentiments from 1 (not at all) to 4 (very much). The inventory was validated for the local population in a previous study [21].

## Study procedure

A free aromatherapy workshop was advertised on social media to attract attendees. One hundred four individuals engaged in this inquiry from January to February 2024. The inclusion criteria comprised adult male and female volunteers. Furthermore, they were mandated to possess a minimum of 20 on both STAI scales. The exclusion criteria included people who had previously practiced meditation, tai chi, or yoga, had received psychiatric or pharmacological therapy, were pregnant, had flu symptoms, or suffered anosmia owing to COVID-19. Ninety-six participants were randomly allocated to each group via a random number table, with the randomization executed by an individual not directly associated with the study. Following group selection, a fundamental questionnaire assessing sociodemographic factors was administered to profile the individuals and the STAI (pretest). The session elucidated the advantages of aromatherapy and essential oils, supplying participants with the oils for a real demonstration. Subsequently, each participant received a weekly aromatherapy kit containing all necessary oil application materials

and an instructional handbook. Nevertheless, those in the EG received kits with peppermint essential oil. Conversely, the participants in the control group were administered the placebo kit, which consisted of a commercial shampoo (Johnson's® baby shampoo, devoid of sulfates and parabens). Every kit was sent to all individuals weekly until the research ended. Furthermore, weekly online sessions were conducted to assess applications and offer comments. The application of essential oils (EOs) adhered to the approach established by Nematollahi et al. [22], with minor alterations, wherein participants were instructed to dispense two drops of EOs or a placebo onto a cotton ball utilizing a dropper. The cotton ball was positioned beneath the participant's nose as they closed their eyes and inhaled deeply 10 times. The cotton was affixed to the participant's collar for 30 minutes. Subsequently, the individual removed and discarded the cotton ball. The applications occurred daily for eight weeks. It was recommended that applications occur at night, five minutes before bedtime, and upon awakening. Upon conclusion of the intervention, the posttest was administered utilizing the identical protocols employed during the pretest (Fig. 1). All participants were apprised of the objectives of the research program and signed a permission form, ensuring confidentiality and anonymity. The Institutional Review Board (IRB) approved the study protocol. This experiment was conducted following the Declaration of Helsinki.

## Data Analysis

The sociodemographic and clinical data of participants were displayed as the mean ± standard deviation (SD). Differences in sociodemographic and clinical characteristics between groups were examined using the Pearson Chi-Square, Likelihood-ratio, and Fisher's exact tests. For the primary study outcomes (anxiety scores in pretest and posttest), the data did not conform to a normal distribution; therefore, they were reported as medians and interquartile ranges (IQRs). Non-parametric

**Table 1. Main chemical constituents (%) of the essential oils of *peppermint*.**

| N° | Compounds | RI | % |
|---|---|---|---|
| 1 | α-pinene | 921 | 0.46 |
| 2 | β-pinene | 965 | 0.51 |
| 3 | Myrcene | 979 | 0.11 |
| 4 | Phellandrene | 993 | 0.12 |
| 5 | p-Cymene | 1011 | 0.20 |
| 6 | Limonene | 1025 | 3.46 |
| 6 | γ-terpinene | 1060 | 0.35 |
| 7 | Linalool | 1094 | 11.68 |
| 8 | Menthone | 1158 | 19.24 |
| 9 | Menthofuran | 1172 | 8.40 |
| 10 | Menthol | 1190 | 42.56 |
| 11 | Terpinen-4-ol | 1201 | 0.22 |
| 12 | α-Terpineol | 1213 | 0.10 |
| 13 | β-Caryophyllene | 1270 | 2.17 |
| 14 | β-Bourbonene | 1284 | 0.25 |
| 15 | Trans-β-farnesene | 1346 | 0.83 |
| 16 | Germacrene D | 1365 | 1.14 |
| 17 | Caryophyllene oxide | 1431 | 0.11 |
| 18 | Bicyclogermacrene | 1447 | 0.12 |
| 19 | γ-Cadinene | 1495 | 0.20 |
| 20 | Ledol | 1584 | 2.16 |
| 21 | Viridiflorol | 1598 | 1.29 |
| 22 | α-Eudesmol | 1622 | 0.15 |
| 23 | β-Eudesmol | 1650 | 0.58 |
| | Total identified (%) | | 96.39 |

RI:Retention index

**Table 2.** Socio-demographic and clinical data of participants .

| Socio-demographic data | CG | EG | Total | p-Value |
|---|---|---|---|---|
| **Gender** | | | | |
| Male | 16 (34.8%) | 18 (38.3%) | 34 (36.6 %) | 0.725[a] |
| Female | 30 (65.2%) | 29 (61.7%) | 59 (63.4 %) | |
| **Age** | | | | |
| Young adults | 3 (6.5%) | 2 (4.2%) | 5 (5.4%) | |
| Middle adults | 27 (58.7%) | 31 (66.0%) | 58 (62.4%) | 0.741[b] |
| Mature adults | 16 (34.8%) | 14 (29.8%) | 30(32.2%) | |
| **Marital status** | | | | |
| Married | 25 (54.3%) | 23(48.8%) | 48 (51.7%) | |
| Unmarried | 15 (32.6%) | 20 (42.6%) | 35 (37.6 %) | 0.483[b] |
| Divorced | 5 (10.9%) | 2 (4.3%) | 7 (7.5%) | |
| Widower | 1(2.2%) | 2(4.3%) | 3 (3.2%) | |
| **Clinical treatment provided** | | | | |
| Psychological | 5 (10.9%) | 4 (8.5%) | 9 (9.7%) | 0.740[c] |
| Pharmacological | 0 (0.0%) | 0(0.0%) | 0 (0.0%) | |
| None | 41 (89.1%) | 43 (91.5%) | 84(90.3%) | |

[a]p-value is calculated by Pearson Chi-Square test.
[b]p-value is calculated by Likelihood-ratio test
[c]p-value is calculated by Fisher's exact test

**Table 3.** Group differences of anxiety variable according to State-Trait Anxiety Inventory (STAI).

| Groups | Pretest | | Posttest | | p-Value[b] |
|---|---|---|---|---|---|
| | Median | IQR | Mean | IQR | |
| **CG** | | | | | |
| State Anxiety | 33.00 | (29.00, 38.00) | 35.00 | (30.00, 40.00) | 0.124 |
| Trait Anxiety | 30.00 | (28.25, 33.00) | 30.00 | (28.00, 35.00) | 0.506 |
| **EG** | | | | | |
| State Anxiety | 35.00 | (31.25, 39.00) | 30.00 | (26.25, 33.75) | 0.001* |
| p-value[a] | 0.137 | | 0.000* | | |
| Trait Anxiety | 30.50 | (27.00, 33.75) | 27.00 | (25.00, 30.00) | 0.001* |
| p-value[a] | 0.935 | | 0.000* | | |

*p<0.05
[a]p-value is calculated by the Whitney U test between groups
[b]p-value is calculated by the Wilcoxon test between study phases

**Table 4.** Fixed Effects from the Mixed Model Analysis Predicting Post-Intervention Anxiety (State Anxiety Post).

| Effect | Numerator df | Denominator df | F-value | p-value |
|---|---|---|---|---|
| Intercept | 1 | 70 | 1562.59 | 0.001 |
| Group (Experimental vs. Control) | 1 | 70 | 16.508 | 0.001 |
| State anxiety pretest | 24 | 70 | 1.504 | 0.096 |

tests were conducted to analyze differences: the Mann-Whitney U test was used to assess statistically significant differences between groups, while the Wilcoxon signed-rank test was used to evaluate differences within groups across study phases. A significance level of p < 0.05 was considered statistically significant. Additionally, mixed models were conducted to assess the fixed effects of group (experimental vs. control) and pretest scores on posttest outcomes. Statistical significance for these models was determined using F-tests, and 95% confidence intervals were calculated for the parameter estimates. To further quantify the impact of the intervention, effect sizes were calculated. SPSS v. 27.0 (IBM Corp., Armonk, NY, USA) was utilized for statistical analysis.
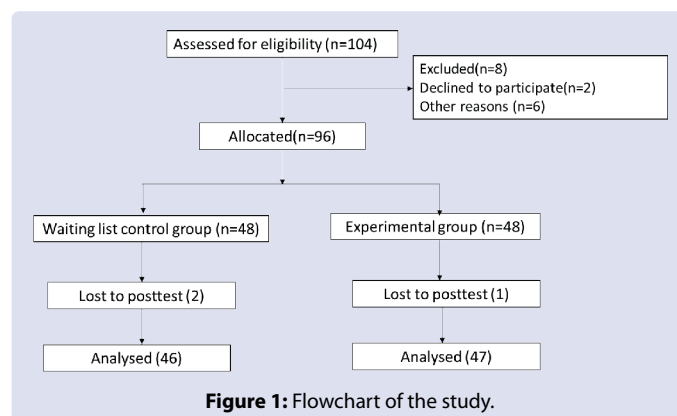


**Figure 1:** Flowchart of the study.

## RESULTS

The primary chemical components of peppermint essential oil are summarized in Table 1, where the total identified constituents represent 96.39% of the essential oil. The analysis suggests that Menthol is the most prevalent compound, comprising 42.56% of the oil. Menthone follows this at 19.24% and Linalool at 11.68%. A substantial quantity of menthofuran is also present, accounting for 8.40%. Other components, including β-Caryophyllene (2.17%), Limonene (3.46%), β-Caryophyllene (2.17%), and Ledol (2.16%), are present in smaller quantities.

Table 2 delineates individuals' socio-demographic and clinical characteristics in the control group (CG) and the experimental group (EG). The gender distribution was comparable across groups, with men representing 36.6% and females 63.4% of the total sample (p = 0.725). Besides, the age distribution of participants was categorized according to developmental stages based on Erikson's theory. The Young Adult group (18–29 years) comprised 5.4% of the total sample (CG: 6.5%, EG: 4.2%), the Middle Adult group (30–39 years) represented 62.4% of participants (CG: 58.7%, EG: 66.0%), and the Mature Adult group (40–50 years) accounted for 32.2% of the sample (CG: 34.8%, EG: 29.8%). No significant differences were found between groups regarding age distribution (p = 0.741). Regarding marital status, 51.7% of participants were married, 37.6% were single, 7.5% were divorced, and 3.2% were widowed, indicating no significant difference between groups (p = 0.483). In terms of clinical treatment, the majority of participants (90.3%) had not received any treatment, while 9.7% had received psychological treatment. Pharmacological treatment was not administered to any participants, and there was no statistically significant difference between the groups in terms of clinical treatment (p = 0.740).

In Table 3, the results of the State-Trait Anxiety Inventory (STAI) for both State Anxiety and Trait Anxiety are presented, comparing the Control Group (CG) and Experimental Group (EG) across study phases. The median values and interquartile ranges (IQR) were determined, and statistical comparisons were made using the Mann-Whitney U test for differences between groups and the Wilcoxon test for changes within groups across the study phases. For State Anxiety, the pretest phase showed no significant difference between the groups, with a median of 33.00 (IQR = 29.00–38.00) in the CG and 35.00 (IQR = 31.25–39.00) in the EG (p = 0.137). However, in the posttest phase, the EG demonstrated a significant reduction in State Anxiety, with a median of 30.00 (IQR = 26.25–33.75) compared to the CG's median of 35.00 (IQR = 30.00–40.00), resulting in a statistically significant difference (p = 0.001). Furthermore, within-group analysis revealed a significant decrease in State Anxiety from pretest to posttest within the EG (p = 0.000), while no significant change was observed in the CG (p

**Table 5. Fixed Effects from the Mixed Model Analysis Predicting Post-Intervention Trait Anxiety (Trait Anxiety Post).**

| Effect | Numerator df | Denominator df | F-value | p-value |
|---|---|---|---|---|
| Intercept | 1 | 77 | 1838.498 | 0.001 |
| Group (Experimental vs. Control) | 1 | 77 | 9.091 | 0.003 |
| Trait anxiety pretest | 17 | 77 | 1.721 | 0.057 |

**Table 6. Effect Size Calculation for State and Trait Anxiety Based on Wilcoxon Test Results.**

| Variable | Z-score | Sample size (N) | Effect size (r) | Effect Interpretation |
|---|---|---|---|---|
| State anxiety | -4.903 | 93 | 0.508 | Large |
| Trait anxiety | -3.293 | 93 | 0.342 | Moderate |

= 0.124). Similarly, Trait Anxiety did not show a significant difference between groups in the pretest phase, with the CG having a median of 30.00 (IQR = 28.25–33.00) and the EG a median of 30.50 (IQR = 27.00–33.75) (p = 0.935). In the posttest phase, the EG exhibited a significant reduction in Trait Anxiety, with a median of 27.00 (IQR = 25.00–30.00) compared to the CG, which maintained a median of 30.00 (IQR = 28.00–35.00), indicating a statistically significant difference (p = 0.001). Additionally, the within-group analysis showed a significant decrease in Trait Anxiety within the EG from pretest to posttest (p = 0.000), while no significant change was noted in the CG (p = 0.506). Consequently, our data demonstrate that the intervention administered to the Experimental Group led to substantial decreases in both State and Trait Anxiety during the post-test phase. Conversely, the Control Group exhibited no significant alterations in anxiety levels throughout the study phases.

Table 4 displays the fixed effects of the mixed model analysis that predict post-intervention state anxiety. The study includes three fixed effects: the intercept, group (Experimental vs. Control), and State Anxiety pretest scores. The intercept was significant, with an F-value of 1562.59 (p = 0.001), indicating a strong baseline level for State Anxiety. Additionally, the effect of the group was significant, with an F-value of 16.508 (p = 0.001), suggesting a significant difference in post-intervention State Anxiety levels between the Experimental and Control groups. This result supports the effectiveness of the intervention in reducing State Anxiety in the Experimental group. In contrast, the pretest State Anxiety scores did not significantly predict post-intervention State Anxiety, as indicated by an F-value of 1.504 and a p-value of 0.096. This suggests that baseline anxiety levels did not significantly influence the post-intervention outcomes, reinforcing the impact of the intervention itself rather than pre-existing anxiety levels on the observed results.

Table 5 presents the fixed effects from the mixed model analysis predicting post-intervention Trait Anxiety. The analysis includes the intercept, group (Experimental vs. Control), and Trait Anxiety pretest scores. The intercept was statistically significant, with an F-value of 1838.498 (p = 0.001), indicating a strong baseline level for Trait Anxiety. The group effect was also significant, with an F-value of 9.091 (p = 0.003), suggesting a significant difference in post-intervention Trait Anxiety between the Experimental and Control groups. This finding supports the effectiveness of the intervention in reducing Trait Anxiety in the Experimental group. Besides, Trait Anxiety scores did not substantially predict post-intervention Trait Anxiety, evidenced by an F-value of 1.721 and a p-value of 0.057, which does not reach the conventional threshold for statistical significance. This result indicates that baseline Trait Anxiety levels did not significantly influence post-intervention outcomes, underscoring the impact of the intervention itself rather than initial anxiety levels on the reduction in Trait Anxiety.

Finally, table 6 presents the effect size calculations for state and trait anxiety following the intervention, based on the Wilcoxon signed-rank test results for within-group comparisons (pretest vs. posttest) in the experimental group. Given a final sample size of 93 participants (46 in the control group and 47 in the experimental group), effect sizes were recalculated to account for participant attrition. The effect size, denoted as "r", was computed using the formula $r = [Z]/\sqrt{N}$, where Z is the Z-score from the Wilcoxon test, and N is the total sample size. The results indicate a large effect size for state anxiety ($r = 0.508$), suggesting a substantial reduction in state anxiety levels post-intervention. For trait anxiety, the effect size was moderate (r = 0.342), indicating a more moderate impact of the intervention on trait anxiety. According to conventional interpretation thresholds, values of r≥0.5 denote a large effect, values around 0.3 a moderate effect, and values around 0.1 a small effect. This analysis confirms that the intervention was particularly effective in reducing state anxiety, with a comparatively moderate effect on trait anxiety.

## DISCUSSION

Our findings align with prior research indicating that menthol, menthone, and menthofuran are among the primary components[23,24]. Menthol ranges from 30.35% to 69.1% in different studies, indicating its dominant presence in peppermint oil[25]. The menthone was found in concentrations between 16.04% and 30.1%, contributing to the oil's characteristic scent and therapeutic properties[26]. Additionally, linalool was identified as a main component, consistent with a study conducted in southern Peru, where it was also reported as one of the principal components[27]. A combination of genetic, ambient, and agronomic factors can be attributed to the similarity in the chemical composition of MPEO , particularly in compounds such as menthol, menthone, and linalool. Primarily, the plant's capacity to synthesize specific compounds in greater proportions is determined by genetic variations and the presence of distinct chemotypes within the species; for instance, certain chemotypes naturally generate higher concentrations of menthol and menthone[28].

Other factors that can influence the chemical composition of peppermint essential oils include harvesting time, plant maturity, extraction methods, and environmental stresses. For instance, the stage of plant growth at the time of harvest can significantly affect the essential oil yield and composition; plants harvested at full bloom often exhibit higher concentrations of menthol and menthone[29]. Environmental factors like soil nutrient availability, water stress, temperature fluctuations, and light exposure can also impact the biosynthesis of essential oil components in peppermint plants, affecting both yield and composition[30].

Most participants were middle-aged and mature adults. This population exhibits a notable inclination towards complementary and alternative medicine (CAM), influenced by both perceived benefits and skepticism towards conventional healthcare. This demographic often seeks CAM for various reasons, including the management of chronic conditions and a desire for holistic health approaches[31]. In addition, dissatisfaction with conventional healthcare, particularly related to cost and waiting times, also drives CAM usage, as well as social networks and psychological factors further contribute to the adoption of CAM in these age groups[32,33]. Besides, most participants were women. This can be related to the fact that women often exhibit a heightened awareness of health-related issues, which can lead to adaptive coping strategies in comparison to men[34].

Based on our findings, EG exhibited substantial reductions in anxiety levels in the post-test phase. Specifically, the effect size for state anxiety reached the threshold for a large effect, indicating a pronounced immediate impact of the intervention on participants' situational anxiety. In contrast, the effect size for trait anxiety, although

significant, was moderate, suggesting that while the intervention also influenced participants' baseline anxiety disposition, the impact was less substantial. Mixed model analysis further supported these results, revealing that group assignment (EG vs. CG) was a significant predictor of post-intervention anxiety outcomes, independent of pretest scores. These results collectively suggest that the intervention was effective in reducing anxiety, with a particularly strong impact on situational or state anxiety.

The noted differential effect of the intervention on state and trait anxiety corresponds with established psychological studies. State anxiety refers to temporary feelings of apprehension or tension in response to specific situations, whereas trait anxiety denotes a more enduring predisposition to perceive situations as threatening[35]. In contrast, trait anxiety, being a stable personality characteristic, typically requires more prolonged and intensive interventions to achieve substantial change. This distinction is supported by findings that trait anxiety can influence the trajectory of therapeutic outcomes, indicating that individuals with higher trait anxiety may need tailored approaches for effective treatment[36].

To support our findings, the literature reports various studies where the potential of MPEO is observed[37–40]. In addition, some components of MPEO exert anxiolytic properties, such as menthol, whose anxiolytic properties have been explored in various studies, though results are mixed. While some research indicates that menthol may have beneficial effects on mood and anxiety[41–43], other studies show limited efficacy[44]. In addition, linalool, a monoterpene found in various essential oils, has demonstrated significant anxiolytic potential in animal[45,46] and human studies[47,48]. Research indicates that linalool exerts its effects through GABAergic and glutamatergic mechanisms, facilitating anxiety reduction without motor impairment[49]. Another component with anxiolytic activity is limonene, a natural monoterpene exhibiting significant anxiolytic potential, as evidenced by various animal[50–52] and human model studies[53].

The anxiolytic effects of MPEO observed in our study may be partially explained by the interaction of its components, such as linalool and menthol, with the GABAergic system[54–56]. However, more evidence is needed to confirm its efficacy and understand the underlying mechanisms.

A primary limitation of our study is the lack of direct biochemical analysis to confirm GABAergic involvement in the anxiolytic effects of MPEO. Additionally, the study relied on self-reported measures, which may be subject to bias. Besides, the sample size, while sufficient to detect significant differences, may limit the generalizability of the findings to broader populations.

## CONCLUSION

Our findings suggest that peppermint essential oil may be an effective intervention for reducing state and trait anxiety, with a more pronounced effect on state anxiety. While the probable mechanism involves modulation of the GABAergic system, further research is required to establish the precise biochemical pathways involved. These results support the potential of peppermint aromatherapy as a complementary treatment for anxiety, particularly for situational anxiety, and underscore the need for additional studies to verify these effects at a neurochemical level.

## CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

## ACKNOWLEDGMENTS

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