Effects of *Melissa officinalis* essential oil on state and trait anxiety

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ABSTRACT

Introduction: Essential oils are complex substances used extensively in aromatherapy and phytotherapy, some of them as anxiety and stress-relieving agents. In order to evaluate the effects of *Melissa officinalis* essential oil on state and trait anxiety, this study was conducted. Methods: The essential oil was extracted by steam distillation and the chemical composition was investigated by Gas chromatography-mass spectrometry (GC–MS). In addition, a Quasi experimental study with measures at pretest-posttest was conducted, where 58 participants were divided into two groups, a waiting-list (WL) control group, and an experimental group (EG) treated with aromatherapy based on *Melissa officinalis essential oil*. The anxiety index was evaluated by State-Trait Anxiety Inventory (STAI). Measures were taken at two times: pretest and posttest. Results: The chemical analysis showed that β-cubebene (26.3%), β-caryophyllene (25.4%) and geranial (12.5%) were the main components. Both state and trait anxiety levels decreased in the experimental group during the posttest phase; however, only state anxiety demonstrated statistically significant differences (p<0.05). A moderate size effect was found for state anxiety (d = 0.799; g = 0.797) with 1-β = 0.803; but none for trait anxiety. Conclusion: *Melissa officinalis* essential oil was moderately effective on state anxiety but has no effect on trait anxiety.

Key words: Essential oil, State anxiety, Trait anxiety, *Melissa officinalis*.

INTRODUCTION

The COVID-19 pandemic has spread across the globe, bringing with it a great deal of fear, anxiety, and uncertainty; however, the primary focus has been on physical health and the control of virus transmission, while mental health has been neglected. In fact, historical evidence indicates that infectious disease epidemics are associated with an increase in mental health issues among survivors and affected populations. In this regard, an increase in the prevalence of mental health issues will probably distinguish the post-pandemic era.

The perception of danger causes an emotional, physiological, cognitive, and behavioral state known as anxiety. It can be initiated by the perception of a threat, including threats to future happiness, threats to self-esteem, and threats to the individual’s capacity to make sense of the data of his experience. Anxiety is adaptive if it occurs as a reaction to a threat and allows a person to cope with the encompassing environment. Nevertheless, anxiety becomes pathological when it triggers considerable personal distress and impairs daily functioning.

Psychological and pharmaceutical interventions constitute the current treatment options for anxiety disorders. In fact, the selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs) are deemed the first-line pharmaceuticals. However, delayed onset of action and the co-occurrence of mood disorders can result in non-responders to treatment. Benzodiazepines (BZDs) can be prescribed for their rapid efficacy, however their potential adverse effects and risks of tolerance and dependence should be minimized and reserved for short-term use. Therefore, there is a need for identifying novel pharmacological approaches with anxiolytic efficacy and fewer side effects.

In this order of ideas, aromatherapy based on essential oils (EOs) may be an alternative to cope anxiety. In fact, a meta-analysis of 741 studies found that aromatherapy with various essential oils, among them, lavender, bergamot, *Citrus sinensis*, rose oil, *Roman chamomile*, and heliotropin, could alleviate anxiety, demonstrating short-term benefits and providing valuable insights into the use of aromatherapy as an alternative treatment for reducing anxiety. In addition, a review showed that EOs may be effective for reducing anxiety, stress, and pain levels, and improving sleep quality among adult patients in intensive care units. Besides, a study evaluating the use of aromatherapy with peppermint essential oil on cardiac patients in an emergency department found a significant reduction in anxiety levels following the intervention. concentrating more on our study population, a meta-analysis concluded that aromatherapy inhalation may reduce anxiety in college students. Thus, the present investigation was conducted to evaluate the effect of *Melissa officinalis* essential oil (MOEO) on trait and state anxiety.

MATERIAL AND METHODS

Plant material

The fresh leaves of *Melissa officinalis* were obtained from a local market and verified by Segundo Leiva Gonzales, Biol, and deposited at the Herbarium Antenor Orrego (HAO) of Antenor Orrego University.
Essential oils extraction

Dust was removed from the freshly collected leaves by washing them with distilled water. The samples were then dried with a forced air circulation stove at 40°C for 24 hours. The powdered plant material (100 g) and 1000 mL of distilled water were deposited in a round-bottomed flask and connected to a Cleverg apparatus. After 3 hours of boiling, hydro distillation was completed. Finally, the oil was then dried with anhydrous sodium sulfate and stored in amber glass containers at 4 °C in a refrigerator for future use in experiments.13

Determination of essential oil composition

Gas chromatography–mass spectrometry (GC/MS) was performed using a Hewlett-Packard 6890/5972 GC/MS using a Br-5MS capillary column. GC conditions were the following: The analysis began after a 3-minute solvent delay, continued with 6 C/min over a 50 °C to 300 °C temperature program, and concluded with a 5-minute pause. MS settings were: 230 °C temperature of the source, 150 C temperature of the MS Quad, and 70 eV ionization energy. The discharge rate of helium was 1 mL/min. Finally, computer matching with mass spectra from the NIST0.2 library and retention indices (RI) compared with the literature Adams indices were used to identify the compounds.14

Study design and sample

A Quasi experimental study with measures at pretest-posttest was conducted. 58 participants were divided into two groups of 29 participants, comprising a waiting-list (WL) control group, and an experimental group (EG) treated with aromatherapy based on MOEO.

Instruments

Evaluation of anxiety was conducted using the State-Trait Anxiety Inventory (STAI), which consists of two self-report scales measuring two distinct types of anxiety: state (actual levels of intensity and anxiety states) and trait (selects individuals who vary in their propensity to react to psychological stress with varying degrees of intensity). Both scales contain 20 statements, and respondents assess the intensity of their feelings about each from 1 (not at all) to 4 (extremely) on a four-point scale. The trait section describes how the subjects feel in general, while the state section describes how they feel at a given moment.15,16 In a previous investigation, validation and reliability coefficients for the local population were determined.17

Study procedure

A complimentary aromatherapy course was offered to students at a private university. 65 undergraduates were enrolled, and 58 of them participated in this study between June and July of 2022. Included in the inclusion criteria were students enrolled in the 2022–01 academic semester and those who scored above 20 on both State-Trait Anxiety Inventory scales. Exclusion criteria included previous meditation, tai chi, or yoga practice, psychiatric or pharmaceutical treatment, pregnancy, influenza, and loss of scent for COVID-19. The students were enrolled in two (A and B) sections of the same course. These sections were formed prior to the advent of the researchers, so there was no randomization. A section was designated as EG and B as WL. Following the selection of groups, instruments were administered (pretest). In addition, each participant received a weekly aromatherapy kit containing all the necessary components for oil applications as well as an instruction manual. Besides, each university student received a new kit every week until the investigation was concluded. Additionally, weekly online meetings were conducted to review applications and provide feedback. Participants were instructed to pour two droplets of EOs onto a cotton ball using a dropper, in accordance with the methodology of Reza et al.18 The participant then held the cotton ball under his or her nose while closing his or her eyes and taking 10 deep breaths. The participant’s collar was then fastened with cotton for 30 minutes. The participant then removed the pin and disposed of the cotton ball. The WL control group did not receive any intervention until the conclusion of the EG administration. The applications occurred daily for six weeks. At the conclusion of the intervention, the instruments were given again (posttest). (Fig. 1). All participants were informed of the investigation program’s objectives and required to complete a consent form guaranteeing confidentiality and anonymity. The protocol for the investigation was approved by the Institutional Review Board (IRB). In addition, this investigation was conducted in accordance with the Helsinki Declaration.

Data analysis

The data were displayed as the mean ± standard deviation (SD). Using the Pearson Chi-Square and Likelihood-ratio analyses, differences in the sociodemographic and clinical data of participants were examined. Because the data did not conform to a normal distribution, non-parametric tests were conducted. The Mann-Whitney U test was used to determine statistically significant differences between groups, and the Wilcoxon test was used to determine statistically significant differences between study phases: p <0.05 was considered statistically significant. In addition to statistical power, Cohen’s D and Hedges’ G as well as percentage of change were calculated between the groups for post-test scores. SPSS v. 27.0 (IBM Corp., Armonk, NY, USA) was utilized for the statistical analysis.

RESULTS

Chemical composition of MOEO is depicted in Table 1, where 23 components were identified, representing 98.1% (area percent) of total oil content, among which β-cubebene (26.3%), β- caryophyllene (25.4%), geraniol (12.5%), α-cadinene (5.7%), and neral (4.8%), were the major constituents.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pentyl propanoate</td>
<td>915</td>
<td>0.2</td>
</tr>
<tr>
<td>mircene</td>
<td>980</td>
<td>0.1</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1008</td>
<td>t</td>
</tr>
<tr>
<td>cis-β-ocimene</td>
<td>1024</td>
<td>0.6</td>
</tr>
<tr>
<td>linalool</td>
<td>1096</td>
<td>0.3</td>
</tr>
<tr>
<td>citronellal</td>
<td>1146</td>
<td>1.6</td>
</tr>
<tr>
<td>octyl acetate</td>
<td>1210</td>
<td>t</td>
</tr>
<tr>
<td>neral</td>
<td>1238</td>
<td>4.8</td>
</tr>
<tr>
<td>geraniol</td>
<td>1253</td>
<td>1.1</td>
</tr>
<tr>
<td>methyl citronellate</td>
<td>1261</td>
<td>0.4</td>
</tr>
<tr>
<td>geranial</td>
<td>1272</td>
<td>12.5</td>
</tr>
<tr>
<td>methyl geranate</td>
<td>1334</td>
<td>0.3</td>
</tr>
<tr>
<td>alpha-Copaene</td>
<td>1390</td>
<td>2.9</td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>1436</td>
<td>25.4</td>
</tr>
<tr>
<td>α-caryophyllene</td>
<td>1465</td>
<td>3.6</td>
</tr>
<tr>
<td>allo-aromadendrene</td>
<td>1491</td>
<td>0.7</td>
</tr>
<tr>
<td>β-cubebene</td>
<td>1512</td>
<td>26.3</td>
</tr>
<tr>
<td>α-muurolel</td>
<td>1523</td>
<td>2.1</td>
</tr>
<tr>
<td>γ-cadinene</td>
<td>1535</td>
<td>0.4</td>
</tr>
<tr>
<td>α-cadinene</td>
<td>1558</td>
<td>5.7</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1594</td>
<td>1.9</td>
</tr>
<tr>
<td>Caryophyllene oxide</td>
<td>1616</td>
<td>3.7</td>
</tr>
<tr>
<td>α-cadinol</td>
<td>1695</td>
<td>3.5</td>
</tr>
<tr>
<td>Total identified (%)</td>
<td></td>
<td>98.1</td>
</tr>
</tbody>
</table>

RI, Retention index; t= traces (<0.1%)
Table 2: Socio-demographic and clinical data of participants.

<table>
<thead>
<tr>
<th>Socio-demographic data</th>
<th>WL</th>
<th>EG</th>
<th>Total</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>12 (46.2%)</td>
<td>10 (35.7%)</td>
<td>22 (40.7 %)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14 (53.8%)</td>
<td>18 (64.3%)</td>
<td>32 (59.3%)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>18-28</td>
<td>21 (80.8%)</td>
<td>25 (89.3%)</td>
<td>46 (85.2%)</td>
</tr>
<tr>
<td></td>
<td>29-38</td>
<td>5 (19.2%)</td>
<td>3 (10.7%)</td>
<td>8 (14.8%)</td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>2 (7.7%)</td>
<td>1 (3.6%)</td>
<td>3 (5.6%)</td>
</tr>
<tr>
<td></td>
<td>Unmarried</td>
<td>24 (92.3%)</td>
<td>27 (96.4%)</td>
<td>51 (94.4%)</td>
</tr>
<tr>
<td></td>
<td>Divorced</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Clinical treatment</td>
<td>Psychological</td>
<td>4 (15.4%)</td>
<td>3 (10.7%)</td>
<td>7 (13.0%)</td>
</tr>
<tr>
<td></td>
<td>Pharmacological</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>22 (84.6%)</td>
<td>25 (89.31%)</td>
<td>47 (87.9%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>p-value is calculated by Pearson Chi-Square test  
<sup>b</sup>p-value is calculated by Likelihood-ratio test

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pretest</th>
<th>SD</th>
<th>Posttest</th>
<th>SD</th>
<th>p-Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL</td>
<td>State Anxiety</td>
<td>35.48 ±6.07</td>
<td>35.65 ±6.05</td>
<td>0.826</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trait Anxiety</td>
<td>26.66 ±5.81</td>
<td>26.77 ±5.67</td>
<td>0.701</td>
<td></td>
</tr>
<tr>
<td>EG</td>
<td>State Anxiety</td>
<td>35.76 ±6.21</td>
<td>30.50 ±6.81</td>
<td>0.007&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trait Anxiety</td>
<td>26.72 ±6.43</td>
<td>25.64 ±5.72</td>
<td>0.305</td>
<td></td>
</tr>
</tbody>
</table>

<sup>*</sup>p<0.05  
<sup>a</sup>p-value is calculated by Mann Whitney U test between groups  
<sup>b</sup>p-value is calculated by Wilcoxon test between study phases

Table 4: Cohen’s d and pretest–posttest percentages of change in intervention groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cohen’s d</th>
<th>Hedges’ g</th>
<th>1-β</th>
<th>% of change Pretest–Postest</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td>State Anxiety</td>
<td>0.799</td>
<td>0.797</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>Trait Anxiety</td>
<td>0.198</td>
<td>0.198</td>
<td>0.107</td>
</tr>
</tbody>
</table>

Figure 1: Flowchart of the study
The socio-demographic and clinical data of the participants who were analyzed are presented in Table 2, where 22 were male and 32 were female. 12 (46.2%) men and 14 (53.8%) women comprised WL, while 10 (40.7%) men and 18 (64.3%) women comprised EG. The majority of participants (n = 46; 85.2%) were between the ages of 18 and 28, with those between the ages of 29 and 38 coming in second (n = 8; 14.8%). Regarding their marital status, 51 (9.4%) were single, 3 (5.6%) were married, and nobody was divorced. Besides, most participants (n = 47; 87.9%) never received treatment, while seven (13.1%) received psychological treatment, and no one received pharmacological treatment from a psychiatrist.

Table 3 displays the mean score and standard deviations for anxiety based on the STAI, where EG does not differ from WL in terms of pretest scores (p>0.05 for state and trait anxiety); however, posttest scores differ only for state anxiety (p<0.05) and not trait anxiety (p>0.05). In addition, anxiety scores show a decrease in posttest study phase (30.50 and 25.64 for State and Trait anxiety respectively) in comparison with pretest (35.76 and 26.72 for State and Trait anxiety respectively) in experimental group (p>0.005 for state anxiety and p>0.05 for trait anxiety). Meanwhile WL show a slight increase in posttest scores compared to pretest scores, but these differences are not statistically significant (p>0.05).

Finally, table 4 shows the scores obtained by the Cohen’s D and Hedges’ G test, as well as the statistical power (1-β) and percentage of change; where scores between 0.5 and 0.8 indicate a moderate size effect, as in the case of state anxiety (d = 0.799; g = 0.797), however scores below 0.20 indicate the absence of an effect as is the case of trait anxiety (d = 0.198; g = 0.198). This table also displays the observed power (1-β), where state anxiety was the only variable with sufficient statistical power (1-β=0.803). It is noteworthy to specify that the standard for the desired power is 0.80. Besides, percentages of change between pretest and posttest measures indicate a decrease in state anxiety of -14.71% and trait anxiety of -4.22%.

**DISCUSSION**

Lemon balm (*Melissa officinalis*) is one of the traditional botanical remedies used to treat gastrointestinal disorders as well as being antispasmodic, sedative, and analgesic, among others. MOEO is an important secondary metabolite of this species and has also been attributed to antibacterial, antifungal, spasmylocytic, and sedative properties. Thus, the chemical composition of MOEO was investigated, revealing β-cubebene (26.3%), β-caryophyllene (25.4%), geranial (12.5%), α-cadinene (5.7%), and nerol (4.8%) as its principal constituents. This partially coincides with an investigation, where the two firsts were also the major components, differing only in the amount of these, and in the fact that geranial was not one of the principal components. Nevertheless, in other research performed in Brazil, the main components were neral and geranial, representing about 80%, however, in our case, the proportion of these compounds was minor. In fact, the components variation is also noted in another research in Andean Chile, where β-caryophyllene and eugenol were the principal components representing about 85%. Indeed, variability in both the content and quality of EOs is affected for considerable variables, for some researchers may be the harvest cut height of plant, time, and number of cut. In addition to investigations on various species of the Lamiaceae family, environmental factors such as humidity, temperature, and altitude of growth location influence the composition of Eos.

Other authors consider to genotype, drying and extraction methods as well as fertilization. Although there is a need for studies that take into consideration the genetic, environmental, and geographical factors present in Andean nations such as Peru to pinpoint the causes of MOEO variation in this region.

Regarding socio-demographic and clinical data, the majority of participants were youthful, unmarried, female students who did not receive pharmacological treatment. This is consistent with a previous study conducted by our research team in which participants shared comparable demographic characteristics. In addition, a study demonstrates that university students have a positive view of complementary and alternative medicine, particularly herbal medicine. However, this is one of the limitations, as the results cannot be generalized without a larger age range and sample size.

Regarding to the anxiolytic properties, in our experiments, both trait and state anxiety were found to diminish. Indeed, percentages of change between pretest and posttest measures indicate a decrease in state anxiety of -14.71% and trait anxiety of -4.22%. However, statistical differences between groups and study phases were only observed for state anxiety. In fact, literature reports the effectiveness of MOEO in reducing anxiety in clinical populations such as cardiac patients or patients undergoing coronary artery bypass surgery. In this sense, hospitalization or surgery conditions may activate a temporal anxiety. At this point, it is pertinent to note that state anxiety is related to temporary situations that change every moment, and when they vanish, the individual no longer experiences anxiety; trait anxiety, on the other hand, is related to specific and permanent characteristics of individuals’ personalities. In effect, state anxiety elevates prior to surgery and use to decline after. Besides, our results are also consistent with a previous investigation where MOEO demonstrated to be more effective against the state anxiety than trait anxiety. Furthermore, in vitro assays and in vivo experiments in animal models also suggest that MOEO modulate anxiety related symptoms.

In addition, we discovered a moderate effect size for state anxiety, which is supported by an adequate statistical power; however, there is no effect for trait anxiety, and statistical power is insufficient. This is probably attributable to the small sample size, another limitation of this study; consequently, these findings should be interpreted with caution and cannot be generalized. In this regard, anamethorapy interventions had demonstrated a moderate to large effect size on anxiety reduction. Therefore, evidence suggests that in individuals without a psychiatric history, various types of essential oils could alleviate anxiety, particularly temporary anxiety, or state anxiety.

Regarding mechanisms of action imparted by EOs, these may act on the benzodiazepine system by modulating the GABA A receptor complex or by activating the 5-HT 1A receptor; as is the case with some major components of MOEO such as geranial (citral), where anxiolytic effect occurs via GABA A and 5-HT 1A receptor modulation. Moreover, an *in silico* study revealed that α-cadinene had a higher docking score than other compounds against the human serotonin receptor, which is associated with mood and anxiety regulation. Others *in silico* studies showed that β-caryophyllene, α-Cubebene and α-cadinol act on key targets, such as CNR2 (Cannabinoid Receptor 2), ADRA2B (Adrenoceptor Alpha 2B), and ADORA2A (Adenosine A2a Receptor), implicated in anxiety disorder. Besides, a research revealed that anxiolytic action of components such as β-caryophyllene (BCP) are mediated through CB 2, a cannabinoid receptor which is involved in the regulation of emotional behaviors including anxiety, therefore the cannabinoid system may play a role in the anxiolytic-like effects of BCP. Thus, MOEO could regulate anxiety through multiple signaling pathways, underscoring its multi-component function. However, this hypothesis is not conclusive and further studies are needed to accurately determine the mechanisms of action of EOs.

**CONCLUSION**

*Melissa officinalis* essential oil exerts a moderate effect against state anxiety but no effect against trait anxiety.
CONFLICTS OF INTEREST
All authors have no conflicts of interest to declare.

REFERENCES


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