

# Anxiolytic-Like Effect of *Cymbopogon Citratus* (Lemongrass) Essential Oil

Paul Alan Arkin Alvarado-García<sup>1,\*</sup>, Marilú Roxana Soto-Vásquez<sup>2</sup>, Luis Enrique Rosales-Cerquin<sup>3</sup>, Santiago M. Benites<sup>4</sup>, Taniht Lisseth Cubas-Romero<sup>1</sup>, Emilio German Ramírez-Roca<sup>5</sup>

Paul Alan Arkin Alvarado-García<sup>1,\*</sup>,  
Marilú Roxana Soto-Vásquez<sup>2</sup>, Luis  
Enrique Rosales-Cerquin<sup>3</sup>, Santiago  
M. Benites<sup>4</sup>, Taniht Lisseth Cubas-  
Romero<sup>1</sup>, Emilio German Ramírez-  
Roca<sup>5</sup>

<sup>1</sup>Escuela de Psicología. Universidad Autónoma del Perú. Lima, PERÚ.

<sup>2</sup>Facultad de Farmacia y Bioquímica. Universidad Nacional de Trujillo, Trujillo, PERÚ.

<sup>3</sup>Hospital de Apoyo Chepén, Chepén, PERÚ.

<sup>4</sup>Universidad Autónoma del Perú. Lima, PERÚ.

<sup>5</sup>Escuela de Farmacia y Bioquímica. Universidad Nacional de San Cristóbal de Huamanga, PERÚ.

## Correspondence

Paul Alan Arkin Alvarado-García

Escuela de Psicología. Universidad Autónoma del Perú. Lima, PERÚ.

E-mail: palvaradog@autonoma.edu.pe

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## ABSTRACT

**Introduction:** Essential oils are complex substances that are widely utilized in the practices of aromatherapy. Certain essential oils are recognized for their potential to alleviate anxiety symptoms. This research was conducted to evaluate the effects of *Cymbopogon citratus* essential oil on anxiety. **Methods:** The chemical composition of the extracted essential oil was analyzed using Gas chromatography with flame-ionization detection (GC-FID) and Gas chromatography-mass spectrometry (GC-MS). In addition, an experimental study with measures at pretest-posttest was conducted, where 128 participants were divided into two groups, a waiting-list (WL) control group, and an experimental group (EG) treated with aromatherapy based on *Cymbopogon citratus* essential oil. The anxiety index was evaluated by Zung Self-Rating Anxiety Scale (SAS). **Results:** The chemical analysis identified geranial (52,1%) and neral (35,2%) as the principal constituents. Anxiety levels decreased in the experimental group during the posttest phase ( $p < 0.05$ ). Besides a large effect size was found for anxiety ( $d = 0.962$ ;  $\Delta = 0.988$ ) with  $1 - \beta = 0.999$  a percentage of change of -10.99%. **Conclusion:** *Cymbopogon citratus* essential oil inhalation showed to be effective in decreasing scores anxiety. Therefore, it has the potential to serve as a supplementary treatment for anxiety.

**Key words:** Essential oil, Anxiety, *Cymbopogon citratus*.

## INTRODUCTION

*Cymbopogon citratus*, popularly known as lemongrass, is a member of the Poaceae family, which comprises over 635 genera and 9000 species.<sup>1</sup> It is native to Asia, Africa, and the Americas and extensively cultivated in both temperate and tropical regions worldwide.<sup>2</sup> Due to its pleasant citrus fragrance, this grass has been utilized for decades in the perfumery, cosmetics, and food industries.<sup>3</sup> In regions of Africa and South America, it is used to flavor tea as well in alcoholic and nonalcoholic beverages;<sup>4</sup> in addition to being used as a tranquilizer, diuretic, antipyretic, and anti-inflammatory in Ayurvedic medicine.<sup>5</sup>

Aromatherapy, a branch of phytotherapy that employs essential oils (EOs) for health maintenance, has been a growing popularity in recent years.<sup>6</sup> EOs are composites of numerous organic compounds, whose chemical composition determines their biological activity and fragrance, and are typically administered *via* inhalation, topical absorption, or ingestion.<sup>7,8</sup> In aromatherapy, EOs are primarily used to enhance mood and promote mental well-being in response to various life stressors and their resulting impact on health, including conditions such as anxiety, depression, and stress, as well as physical ailments linked to immune system dysfunction.<sup>9</sup>

Anxiety can be characterized as a state of emotional diffusion that occurs over a period of time and is typically triggered by a situation that may pose a threat to an individual's well-being, although the

likelihood or probability of harm in such situations is often uncertain or low.<sup>10</sup> In this sense, anxiety in and of itself does not constitute a pathological condition, as it may serve as a safeguard against potential harm; nevertheless, if anxiety persists and/or is provoked by stimuli that are not actually threatening, it transforms into maladaptive behavior.<sup>11</sup>

Nowadays, anxiety disorders are treated through psychological and pharmaceutical interventions. In fact, the pharmaceuticals known as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are the primary treatment option; however non-response to treatment may occur due to delayed onset of action and the presence of comorbid mood disorders.<sup>12</sup> Benzodiazepines (BZDs) are often prescribed due to their prompt therapeutic response. Nevertheless, it is imperative to mitigate their potential negative consequences and the likelihood of tolerance and dependence by restricting their use to the short-term.<sup>13</sup> Hence, it is imperative to identify innovative pharmacological strategies that possess anxiolytic properties while exhibiting minimal adverse effects. In this order of ideas, the present investigation was conducted to evaluate anxiolytic-like effect of essential oil from *Cymbopogon citratus* (lemongrass).

## MATERIAL AND METHODS

### Plant material

Fresh leaves of *Cymbopogon citratus* were acquired from a local market, identified by Segundo Leiva Gonzales, Biol, and deposited at the Herbarium Antenor Orrego (HAO) of Antenor Orrego University.

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## Essential oils extraction

The freshly collected leaves were washed with distilled water to remove any dust. The samples were then desiccated for 24 hours in a 40°C oven with forced air circulation. A round-bottomed flask containing 100 g of powdered plant material and 1 liter of distilled water was connected to a Clevenger apparatus. After boiling for three hours, hydro distillation was completed. The oil was then dehydrated with anhydrous sodium sulfate and refrigerated at 4 °C in amber glass containers for future use in experiments.

## Determination of essential oil composition

The essential oil was analyzed by Gas chromatography with flame-ionization detection (GC-FID) and Gas chromatography-mass spectrometry (GC-MS) using DB-5 column. GC was performed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector (FID) under the following analytical conditions: GC oven temperature was maintained at 50 °C for 5 min and programmed to reach 300 °C at a rate of 5 >°C/min, injection temperature was 240 °C, carrier gas hydrogen, flow rate of 35 ml/min. GC-MS was carried out using a Hewlett-Packard 6890 series gas chromatograph coupled with a mass selective detector Hewlett Packard MSD 5972 with a DB-1 column with ionization energy of 70 eV and Helium used as carrier gas at a flow rate of 0.9 ml/min. The identification of essential oil constituents was completed by comparing their GC retention indices (RI) and mass spectra to those in the Wiley library and the NIST 2011 mass spectra library.<sup>14,15</sup>

## Study design and sample

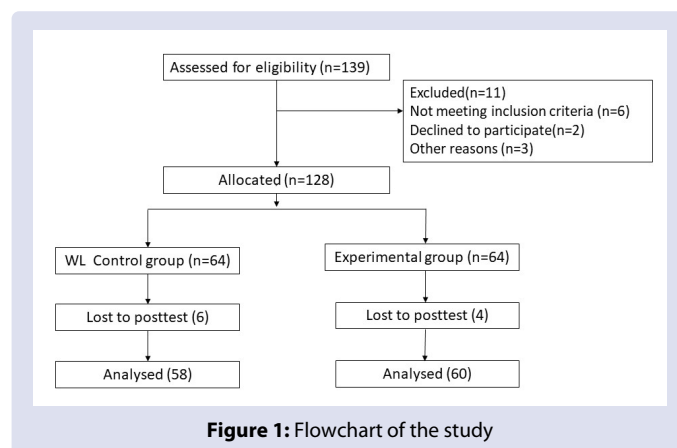
An experimental study with measures at pretest-posttest was conducted. A power analysis was completed by using "G Power 3.1" with a moderate effect size, an  $\alpha$  level of 0.05, and power of 0.80. The number of participants required to determine a difference in effect was 128, 64 per group, comprising a waiting-list (WL) control group, and an experimental group (EG) treated with aromatherapy based on *Cymbopogon citratus* essential oil (CCEO).

## Instruments

Zung Self-Rating Anxiety Scale (SAS): The present scale was devised with the aim of assessing the frequency of symptoms related to anxiety. The scale comprises a total of 20 items, each of which is evaluated on a scale ranging from 1 to 4 (1 = non or a little of the time, 2 = some of the time, 3 = good part of the time, 4 = most of the time)<sup>16</sup> Validation and reliability coefficients for the local population were determined in a previous study.<sup>17</sup>

## Study procedure

A social media platform was utilized to extend an invitation for participation in a complimentary course on aromatherapy. A total of 139 individuals were initially enrolled in the study, with 128 ultimately participating in the investigation during the period spanning from October to December of 2022. The study's inclusion criteria encompassed both male and female participants aged 18-50 who obtained a score exceeding 45 in SAS. Conversely, the exclusion criteria comprised individuals who had previously engaged in alternative therapies like meditation, tai chi, or yoga, received psychiatric treatment, or were pregnant. The study involved the randomization of 64 participants for each group by an independent individual, using a random number table. Upon the formation of WL and EG, a fundamental questionnaire was utilized to characterize participants based on their social-demographic characteristics. Subsequently, the SAS instrument was distributed to all participants for completion as a pretest. Furthermore, every participant was provided with a weekly aromatherapy kit that included all the essential components for oil



applications, along with a manual of instructions. Additionally, each participant was provided with a new kit weekly until the investigation was concluded. Furthermore, regular virtual gatherings were organized to assess submissions and provide feedback. The methodology of Reza *et al*<sup>18</sup>, was followed, whereby the participants were directed to apply two droplets of essential oils onto a cotton ball using a dropper. Subsequently, the participant proceeded to hold the cotton ball beneath their nasal cavity, proceeded to shut their eyes, and inhaled deeply for a total of 10 breaths. The collar of the participant was secured using cotton material for a duration of 30 minutes. Subsequently, the individual extracted the pin and appropriately discarded the cotton ball. The control group assigned to the waitlist condition did not receive any form of intervention until the completion of the experimental group's intervention. The applications were conducted daily over a period of eight weeks. After the intervention, SAS was administered once more as a posttest. (Figure 1). The study's objectives were communicated to all participants, who were then obligated to provide written consent ensuring the preservation of their 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  $\pm$  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 Glass's delta 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

Table 1 illustrates the chemical composition of CCEO, wherein 14 constituents were detected, accounting for 98.9% (area percent) of the entire oil content, among which geranial (52.1%) and neral (35.2%) were identified as the major components.

The socio-demographic and clinical data of the participants who were analyzed are presented in Table 2 displays the socio-demographic and clinical characteristics of the analyzed participants, comprising 43 (36.4%) males and 75 (63.6%) females. The WL group was composed of 23 men, accounting for 39.7% of the group, and 35 women, accounting for 60.3% of the group. On the other hand, the EG group was composed

**Table 1: Main chemical constituents (%) of *Cymbopogon citratus* essential oil.**

Compounds	RI	%
α-thujene	932	1.3
α-pinene	940	0.2
camphene	951	0.3
sabinene	983	0.2
myrcene	995	4.1
limonene	1032	0.6
linalool	1070	0.9
terpinen-4-ol	1185	t
neral	1240	35.2
geraniol	1255	3.5
geranial	1269	52.1
geranyl acetate	1357	0.3
γ-cadinene	1530	t
caryophyllene oxide	1584	0.2
Total identified (%)		98.9

RI, Retention index; t= traces (<0.1%)

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

Socio-demographic data	WL	EG	Total	p-Value
<b>Gender</b>				
Male	23 (39.7%)	20 (33.3%)	43 (36.4%)	0.476 <sup>a</sup>
Female	35 (60.3%)	40 (66.7%)	75 (63.6%)	
<b>Age(yr)</b>				
18-25	25 (43.1%)	22 (36.7%)	47 (39.8%)	0.475 <sup>a</sup>
26-50	33 (56.9%)	38 (63.3%)	71 (60.2%)	
<b>Marital status</b>				
Married	10 (17.2%)	14 (23.3%)	24 (20.3%)	
Unmarried	44 (75.9%)	43 (71.7%)	87 (73.8%)	0.673 <sup>b</sup>
Divorced	4 (6.9%)	3 (5.0%)	7 (5.9%)	
<b>Clinical treatment provided</b>				
Psychological	5 (8.6%)	4 (6.7%)	9 (7.6%)	0.689 <sup>b</sup>
Pharmacological	0 (0.0%)	0(0.0%)	0 (0.0%)	
None	53 (91.4%)	56(93.3%)	109(92.4%)	

<sup>a</sup>p-value is calculated by Pearson Chi-Square test.

<sup>b</sup>p-value is calculated by Likelihood-ratio test.

of 20 men, accounting for 33.3% of the group, and 40 women, accounting for 66.7% of the group. The majority of the participants (60.2%, n = 71) fell within the age range of 26–50, while the rest (39.8%, n = 47) consisted of individuals aged 18–25. The results indicate that there are no statistically significant differences between the two groups of participants with respect to gender and age ( $p > 0.05$ ). In relation to their matrimonial status, a total of 87 individuals (equivalent to 73.8%) reported being unmarried, while 24 individuals (20.3%) were identified as married, and 7 individuals (5.9%) reported being divorced. The majority of the participants (n = 109; 92.4%) did not receive any form of treatment, whereas a small proportion (7.6%) received only psychological treatment, and no one received pharmacological treatment from a psychiatrist. In relation to marital status and clinical treatment, there were no statistically significant differences between the groups ( $p > 0.05$ ).

Table 3 presents the mean score and standard deviations for anxiety measured by the SAS instrument. The results indicate that there is no significant difference between the EG and WL groups in terms of pretest scores ( $p > 0.05$ ). However, there is a statistically significant difference between the groups in terms of posttest scores ( $p < 0.05$ ). Furthermore,

the experimental group exhibited a decline in anxiety scores during the posttest study phase. Specifically, there was a decrease from  $56.9 \pm 7.52$  to  $50.37 \pm 7.28$ , indicating significant statistical differences between the study phases ( $p < 0.05$ ). The results indicate a slight increase in posttest scores compared to pretest scores for WL. However, these disparities do not attain statistical significance ( $p > 0.05$ ).

Finally, table 4 presents the results of the Cohen's D and Glass's delta tests, along with the statistical power ( $1-\beta$ ) and percentage of change. A score greater than 0.8 is indicative of a large effect size, as observed in the case of anxiety ( $d = 0.962$ ;  $\Delta = 0.988$ ). This table also exhibits the observed power ( $1-\beta$ ), with anxiety demonstrating a statistically significant power ( $1-\beta = 0.999$ ), thereby providing support for our findings. It is important to note that the statistical power standard is typically set at 0.80. Furthermore, the analysis of the pretest and posttest measures reveals a reduction in anxiety levels by -10.99%.

## DISCUSSION

Some studies have demonstrated that citral is the primary constituent of CCEO which is comprised of two major compounds, namely geraniol and neral, accounting for over 50% of the total content.<sup>19-21</sup> Our investigation yielded similar results, with citral comprising 87.3% of the total content. On the contrary, other studies show myrcene among other compounds as principal constituents.<sup>15,22</sup> The observed dissimilarities can be attributed to a multitude of factors, encompassing geographic origin, genetic diversity, part of the plant used, maturity level, light exposure, temperature, extraction method and agricultural techniques.<sup>23</sup>

Regarding the sociodemographic and clinical data of participants, our findings align with previous research indicating that women tend to utilize complementary and alternative medicine (CAM) therapies more frequently than men and that young and young adult individuals are more inclined to use these therapies than their older counterparts.<sup>24,25</sup> In fact, historically, women have been socialized to adopt gender roles that emphasize help-seeking and a patient demeanor. These roles have been more closely associated with femininity than masculinity and may contribute to women's greater utilization of healthcare services compared to men;<sup>26</sup> in addition to differences in values and personality traits such as risk-seeking behavior between men and women.<sup>27</sup> In the other hand, young and young adults tend to be more open to trying new things and exploring alternative options. Additionally, younger people may have a greater interest in self-care and health promotion, which could lead them to seek out CAM to improve their overall health

**Table 3: Group differences of anxiety variable according Zung Self-Rating Anxiety Scale (SAS).**

Groups	Pretest		Posttest		p-Value <sup>b</sup>
	Mean	SD	Mean	SD	
<b>WL</b>					
Anxiety	56.48	±7.57	57.20	±6.91	0.470
<b>EG</b>					
Anxiety	56.59	±7.52	50.37	±7.28	0.001 <sup>*</sup>
p-value <sup>a</sup>	0.946		0.001 <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.**

Group	Cohen's d	Glass's delta	1-β	% of change Pretest-Posttest
<b>EG</b>				
State Anxiety	0.962	0.988	0.999	-10.99



and well-being in contrast to older people which may be more likely to have established relationships with conventional healthcare providers and may be less likely to seek out alternative options.<sup>26</sup>

Moreover, the group subjected to the experiment demonstrated a decrease in their anxiety scores during the posttest phase; which is in accordance with a prior study that reported a decrease in state anxiety and subjective tension following the administration of CCEO treatment.<sup>28</sup> Furthermore, a study conducted on women demonstrated that CCEO has the ability to regulate mood by promoting a state of tranquility.<sup>29</sup> Similarly, the efficacy of lemongrass essential oil in mitigating dental anxiety in children was demonstrated through a study, which reported a noteworthy decrease in anxiety levels among the participants.<sup>30</sup> Aforementioned statements are substantiated by research indicating that the anxiolytic properties of lemongrass essential oil are mediated through the GABA<sub>A</sub> receptor–benzodiazepine complex.<sup>31,32</sup> The modulation of anxiety-related behaviors is considerably impacted by GABAergic neurotransmission in the amygdala. It has been observed that the administration of gamma-aminobutyric acid (GABA) or GABA receptor agonists within the amygdala region leads to a decrease in behaviors associated with anxiety.<sup>33</sup>

In fact, benzodiazepines belong to a category of anxiolytic agents that attach to GABA<sub>A</sub> receptors located at the interface between adjacent  $\alpha$  and  $\gamma 2$  subunits, thereby augmenting the flow of GABA-induced chloride ions.<sup>34</sup> This leads to neuronal hyperpolarization and allosteric modulation of these receptors.<sup>35</sup> The chloride ion channels known as GABA<sub>A</sub> receptors are activated by the presence of GABA and can be modulated by various pharmaceutical agents including certain aromatic compounds that can be inhaled such as terpenoids.<sup>32,35-37</sup> Moreover, according to two studies, the anxiolytic effect of the essential oil cannot be attributed to a single component, but rather to the synergy of the main components.<sup>31,32</sup>

Finally, although we found a large effect size and adequate statistical power, the percentage of change only showed a reduction of 10.99%. In fact, one of the limitations of the study is that the sample size is insufficient for generalization. Conversely, the perception and response to odors can be influenced by various individual factors such as genetics, physiology, and psychology, thereby posing a challenge in extrapolating the findings to the general population. Hence, these findings are not conclusive, and further studies are needed to generalize the results.

## CONCLUSION

The study findings suggest that aromatherapy based on lemongrass essential oil may possess anxiolytic properties, as evidenced by a reduction in anxiety scores among participants. As such, it may hold promise as an adjunctive therapy for anxiety treatment.

## CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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## GRAPHICAL ABSTRACT

### Anxiolytic-like effect of *Cymbopogon citratus* (lemongrass) essential oil



#### Group differences of anxiety variable according to Zung Self-Rating Anxiety Scale (SAS)

Groups	Pretest		Posttest		p-Value <sup>b</sup>
	Mean	SD	Mean	SD	
WL					
Anxiety	56.48	±7.57	57.20	±6.91	0.470
EG					
Anxiety	56.59	±7.52	50.37	±7.28	0.001*
p-value <sup>a</sup>	0.946		0.001*		

\*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.

#### Cohen's d and pretest–posttest percentages of change in intervention groups

Group	Cohen's d	Glass's delta	1-β	% of change Pretest-Posttest
EG				
State Anxiety	0.962	0.988	0.999	-10.99

## ABOUT AUTHORS



**M.Ed. Paul Alan Arkin Alvarado García:** Master of Education. Professor at the Medicine School of Universidad César Vallejo. Develops research in Mental health problems, Complementary and Alternative Medicine, and natural products applied to mental disorders.



**Dra. Marilú Roxana Soto-Vásquez:** Doctor in Pharmacy and Biochemistry, professor of the chairs of pharmacognosy and phytochemistry of School of Pharmacy and Biochemistry of Universidad Nacional de Trujillo. Develops Research in Natural Products with antiprotozoal, antitumor, antimicrobial activities, and Complementary and Alternative Medicine.



**MD. Luis Enrique Rosales-Cerquin:** Gynecologist and obstetrics specialist. Hospital de Apoyo-Chepén. Develops research in reproductive health, natural products, and Integrative medicine.



**Dr. Santiago M. Benites:** Doctor in Biomedical Science, Master in Science, researcher at Universidad Autónoma del Perú. Develops Research in Natural Products.



**Dr. Taniht Lisseth Cubas Romero:** Doctor in Psychology. Professor at School of Psychology of Universidad Autónoma del Perú.



**Dr. Emilio German Ramírez-Roca:** Doctor in Pharmacy and Biochemistry, dedicated to the investigation of natural resources and biochemical processes.

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