

Anxiolytic-like Effect of Essential Oils Extracted from *Lippia alba* and *Lippia citriodora*

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ABSTRACT

Introduction: Essential oils from natural plants are widely used around the world to relieve mental conditions. The aim of this investigation was to determine the anxiolytic-like effect of essential oils extracted from *Lippia alba* and *Lippia citriodora*. **Methods:** The oil was extracted by hydro-distillation for 3 hours using a modified Clevenger-type apparatus and then chemical composition was investigated by gas chromatography analysis (GC) and gas chromatography-mass spectrometry (GC-MS). Moreover, a randomized experimental trial was conducted, where 95 participants were divided into 3 groups, comprising a waiting list control group and two experimental groups treated with essential oils by inhalation. The anxiety index was evaluated by State-Trait Anxiety Inventory (STAI). Measures were taken two times: pretest and posttest. **Results:** chemical analysis showed that carvone was the main component (64.5%) for *Lippia alba* essential oil and geraniol (39.8%) for *Lippia citriodora*. State and Trait anxiety scores showed a decrease in posttest study phase in comparison with pretest in all experimental groups ($p < 0.05$). Cohen's D and Hedges' G scores show a large size effect in state anxiety for experimental group treated with *Lippia alba* while moderate changes were found for the rest of variables in all experimental groups. **Conclusion:** the essential oils extracted from *Lippia alba* and *Lippia citriodora* may be useful as a mean to counteract anxiety, specifically state anxiety showing short-term benefits.

Key words: Essential oils, Anxiety, *Lippia alba*, *Lippia citriodora*.

INTRODUCTION

COVID-19 pandemic has caused an unprecedented situation around the world, and one of the most significant consequences is the growing increase of mental health problems¹, even the term coronaphobia was coined to describe the fear and anxiety caused by pandemic², associated to high levels of depression, hopelessness, suicidal ideation, and functional impairment³.

Indeed, anxiety is one of the most prevalent psychological problems during the pandemic⁴. According to a research, the prescriptions of anti-anxiety and sleep aid medication increased for 35.7% and 41.2% of the population respectively, because of the pandemic⁵. It is known that benzodiazepines (BDZs) and antidepressants are widely prescribed for anxiety disorders, nevertheless these drugs can produce side effects as lethargy, retrograde amnesia, drowsiness, dizziness, and vertigo⁶, as well as cause physical dependence and tolerance⁷. Besides, the abrupt cessation of BDZs can cause life-threatening seizures, delirium, and death⁸.

Besides, there is a growing acceptance for Complementary and Alternative Medicine (CAM) treatments, since are perceived as natural, low-risk, economical, with fewer side effects and as effective as conventional drugs⁹. In this context, aromatherapy employs essential oils (EOs), secondary metabolites mainly extracted from leaves, flowers, rhizomes, seeds, barks, and resins of aromatic plants¹⁰.

EOs are isolated via steam distillation, hydrodistillation, solvent extraction, enfleurage,

mechanical pressing, carbon dioxide (CO₂) and supercritical CO₂ extraction, among others¹¹. In aromatherapy EOs are applied through inhalation, massage, compress, and baths; in addition to being used as therapeutic agents and aesthetics¹².

Evidence suggests that EOs could alleviate anxiety significantly showing short-term benefits¹³. Besides, a meta-analysis found that aromatherapy significantly decreased pain and anxiety in the first stage of labor in nulliparous women¹⁴. In this order of ideas, a study examined the effects of rose damascene oil on anxiety and sleep quality in cardiac patients, where this aroma significantly reduced anxiety in the experimental group compared to the control group¹⁵. In addition, an investigation found that aromatherapy based on EOs is a cost-effective method that can reduce anxiety in hospitalized patients¹⁶.

Lippia alba and *Lippia citriodora* are two aromatic shrubs that belong to the Verbenaceae family and have a wide distribution, especially in tropical and subtropical as well as temperate zones of the Americas, Africa, and Asia¹⁷. Traditionally, these species are consumed as tea or essential oil in Latin America and Africa due to their sedative and tranquilizer properties¹⁸.

Despite that *Lippia* species are associated to an anxiolytic effect in traditional medicine and animal models^{19,20}, there is lack of evidence about EOs in human models. In this sense, the objective of this study was to determine the anxiolytic-like effect of essential oils extracted from *Lippia alba* and *Lippia citriodora* in human volunteers.

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MATERIALS AND METHODS

Plant material

The leaves of *Lippia alba* and *Lippia citriodora* were collected from Cajamarca, a city in northern highlands of Peru at 2750 masl. The sample collection was conducted in the months of January to February 2021. Voucher specimens were prepared and identified by Segundo Leiva Gonzales, Biol, and deposited at the Herbarium Antenor Orrego (HAO) of Antenor Orrego University.

Essential oils extraction

The freshly collected leaves were washed with distilled water to remove dust. Then, samples were dried using a forced air circulation stove at a temperature of 40°C for 24 h. Next, leaves were milled, and the powdered plant material (100 g) were placed in a round bottom flask with 1000 ml distilled water, and then connected to a Clevenger-type apparatus. Hydrodistillation was completed for 3 h after boiling. Then the oil was dried over anhydrous sodium sulphate and stored in a refrigerator in amber glass vials at 4°C for further use in experiments²¹. The same procedure was carried out for both species.

Determination of essential oil composition

Gas chromatography analysis (GC) was performed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector (FID), using the following conditions: column temperature, 40 °C (8 min) to 180 °C at 3 °C/min, 180-230 °C at 20 °C/min, 230 °C (20 min); injector temperature 250 °C, detector temperature 250 °C; split ratio 1:50; carrier gas H₂ (34 KPa). Gas chromatography-mass spectrometry (GC-MS) was carried out using a Hewlett-Packard 6890 series gas chromatograph coupled with a mass selective detector Hewlett Packard MSD 5972. The system conditions were the following: fused silica capillary column HP-Innowax (30m x 0.25 mm i.d., 0.25 µm film thickness), column temperature, 40 °C (8 min) to 180 °C at 3 °C/min, 180-230 °C at 20 °C/min, 230 °C (20 min); interface 280 °C; split ratio 1:100; carrier gas He (56 KPa); EI mode 70 eV. Finally, the identification of essential oil constituents was accomplished by visual interpretation, comparing their retention indices and mass spectra with literature data and with those in the NIST 2011 mass spectra library as well as Wiley library²².

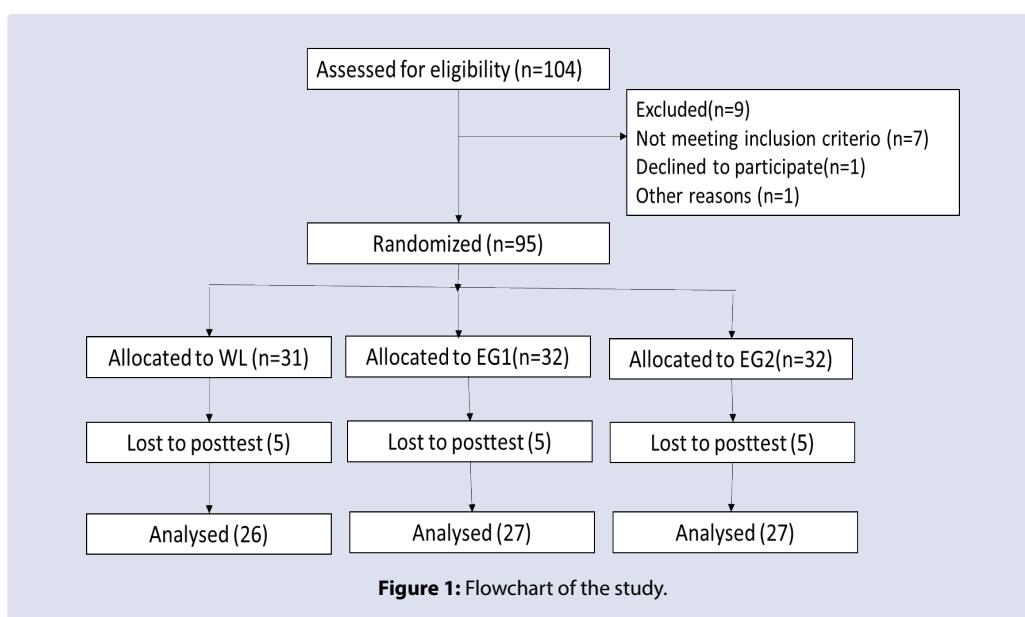
Study design and sample

An experimental study with measures at pretest–posttest was conducted, using two experimental groups and a waiting-list control

group. 95 participants were divided into three groups of 32 participants for each experimental group, the first one treated with aromatherapy based on *L. alba* essential oil (EG1) y the second group treated with *L. citriodora* essential oil (EG2); and finally, a wait-list (WL) control group with 31 participants.

Study procedure

A free aromatherapy course was offered through social media to recruit participants. The course was conducted entirely online, due to government restrictions due to the global pandemic. 104 people were enrolled and 95 took part in this investigation between April and May 2021. Inclusion criteria included participants men and female between the ages of 18-55 and they were required to have a State-Trait Anxiety Inventory score of greater than 20 in both scales; meanwhile exclusion criteria were participants with previous practice of alternative therapies such as meditation, tai chi or yoga, psychiatric or psychological treatment, and pregnancy. Participants for each group were randomized by a person not involved in the study by utilization of a random number table. After groups were formed, instruments were administered (pretest). Besides, each participant received weekly an aromatherapy kit with everything they needed for applications, including an instruction manual. Each kit was sent each week to each participant until the investigation was completed. In addition, weekly online meetings were held to monitor the applications and provide feedback. The EOs applications were according to the methodology of Reza, Bazeli, Basiri and Aalami²³, where the participants were asked to pour two drops of EOs on a cotton ball using a dropper. Next, the cotton ball was held under the participant's nose while closed his or her eyes and took 10 deep breaths. Then, the cotton was pinned to the collar of the participant for 30 minutes. After that, the participant unpinned and disposed the cotton ball. WL control group did not receive any intervention until applications in EG1 and EG2 ended. The applications were every day for 4 weeks. At the end of interventions, an anxiety self-report instrument was administered (posttest). (Fig. 1). When the offered course finished, all participants were informed about the investigation program goals and signed a consent form in which confidentiality and anonymity were guaranteed. The study protocol was approved by Institutional Review Board (IRB) of Universidad Nacional de Trujillo. Besides, this investigation was performed in accordance with the Declaration of Helsinki.



Instruments

Anxiety was evaluated 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 tendency to react to psychological stress with varying degrees of intensity). Both scales consist of 20 statements and respondents rate the intensity of their feelings about each at that moment from 1 (not at all) to 4 (very much so). The part that regards trait describes how the subjects generally feel, while the part that regards state describes how they feel at a given moment^{12,24}. Inventory was validated for local population in a previous study²⁵.

Data Analysis

Data were presented as mean \pm standard deviation (SD). As data did not conform to the normal distribution, Kruskal-Wallis test was used to determine significant differences between groups, while Wilcoxon test was used to determine significant differences between the study phases. Cohen's D, Hedges' G and Percentage Change were calculated between pretest and posttest scores. All statistical analysis was performed using SPSS v.25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Table 1 shows the results from samples analyzed by GC/MS, where 30 components were identified in the essential oil of *L. alba*, representing 98.1% (area percent) of total oil content, among which the major constituents were carvone (64.5%), limonene (6.3%), germacrene D (5.8%), linalool (4.8%), (E)-Nerolidol (2.6%), sabinene (2.4%), α -muurolene(2.3%), piperitenone (1.9%) and neral (1.2%). In the case of the essential oil of *L. citriodora*, 33 components were identified, representing 97.6% (area percent) of total oil content, among which geranial (39.8%), neral (29.1%), limonene (9.6%), germacrene D (3.6%), β -E-ocimene (2.3%), linalool (1.9%), β -caryophyllene (1.8%) and sabinene (1.7%) were the main constituents.

Table 2 presents socio-demographic and clinical data of analyzed participants where 36 (45%) were male and 44 (55%) were female. WL was formed by 12(46%) male and 12(54%) female, while EG1 and EG2 were formed by 12(44%) male and 15(56%) female respectively. Most participants were between 25-35 years old (n=38; 48%), followed by the participants between 36-55 years old (n=32; 40%); and the rest between 18-24 years old (n=10; 12%). In relation to level of education, 42 people (53%) were graduated, 21 (26%) were postgraduate and 17 (21%) were undergraduate. In relation to their marital status, 41(51%) were unmarried, 28 (35%) were married, 6 (8%) were divorced and 5 (6%) participants were widowed.

Table 3 shows the mean score and SDs for anxiety based on STAI, where all groups does not present differences in pretest scores ($p > 0.05$ for state and trait anxiety); somehow, differences are shown in posttest scores ($p < 0.05$ for state anxiety and $p < 0.05$ for trait anxiety). Besides, anxiety scores show a decrease in posttest study phase as EG1 ($p < 0.005$ for state anxiety and $p < 0.05$ for trait anxiety). These results show a change in STAI scores after the intervention. Meanwhile WL show a slight increase in posttest scores in comparison with pretest scores, but it does not show statistically significant differences ($p > 0.05$).

Besides, Table 4 shows the scores obtained by the Cohen's D test, Hedges' G and the percentage of change found; where values between 0.5 and 0.8 show moderate changes, as is the case of trait anxiety in EG1 ($d = 0.66$; $g = 0.66$) and state ($d = 0.78$; $g = 0.78$) and trait ($d = 0.49$; $g = 0.48$) anxiety in EG2, while values higher than 0.80 show a large effect size as evidenced for state anxiety in EG1 ($d = 0.93$; $g = 0.93$). Likewise, the percentages of change showed that state and trait anxiety in EG1 obtained a percentage of change of -14.78%, and -13.18% respectively, in addition, state and trait anxiety in EG2 obtained a percentage of change of -11.67%, and -10.51% respectively.

Table 1: Main chemical constituents (%) of the essential oils of *Lippia alba* and *Lippia citriodora*.

Composition	RI	L. alba	L. citriodora
2-hexenal	848	-	t
α -Thujene	921	0.3	0.1
α -Pinene	928	0.2	1.1
β -citronellene	939	-	t
Sabinene	960	2.4	1.7
1-octen-3-ol	965	0.5	-
β -Pinene	984	0.5	1.0
Myrcene	989	0.4	0.2
3-octanol	996	-	0.1
p-cymene	1021	-	t
Limonene	1026	6.3	9.6
β -E-ocimene	1044	0.2	2.3
Cis-sabinene hydrate	1060	-	0.3
Linalool	1094	4.8	1.9
α -thujone	1110	-	0.5
Trans-limonene oxide	1130	t	0.8
Terpinen-4-ol	1170	-	t
Trans-dihydrocarvone	1178	0.1	-
Carvone	1224	64.5	-
Neral	1240	1.2	29.1
Geranial	1270	0.8	39.8
Piperitenone	1310	1.9	-
δ -elemene	1336	-	0.4
Eugenol	1356	t	t
α -copaene	1373	0.5	0.2
β -bourbonene	1385	0.4	1.1
β -cubebene	1392	0.3	t
β -caryophyllene	1420	0.5	1.8
Aromadendrene	1455	0.4	0.5
Germacrene D	1476	5.8	3.6
α -muurolene	1495	2.3	0.4
γ -cadinene	1508	0.1	0.1
δ -cadinene	1520	0.2	0.3
(E)-Nerolidol	1575	2.6	0.1
α -muurolol	1631	0.1	0.2
α -cadinol	1654	0.1	0.4
(E)-Phytol	2080	0.8	t
Nd	2232	0.1	-
Total identified (%)		98.1	97.6

RI, Retention index; t= traces (<0.1%); Nd, Not determined

DISCUSSION

EOs are metabolites of plants that are typically oily liquids at ambient temperatures. In this case, chemical composition showed that *L. alba* essential oil can be classified as carvone chemotype due to this component is the main according to outcomes. This agrees with data from other investigations where it is also reported this chemotype for Peru^{26,27}. However, *L. citriodora* essential oil showed that geranial, neral and limonene were the compounds where the major quantitative differences were observed. Although it is true that there are no studies on the chemical composition of the essential oil of this species in Peru, this coincides with other studies where they also show these two components as the main ones in the composition of this essential oil^{28,29}.

Regarding socio-demographic data of participants, the majority were adults with graduate and postgraduate level of education. This is in concordance with some studies which affirm that most educated population is more likely to be interested in complementary and

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

Socio-demographic data	WL	EG1	EG2	Total
Gender				
Male	12 (46%)	12 (44%)	12 (44%)	36 (45%)
Female	14 (54%)	15 (56%)	15 (56%)	44 (55%)
Age(yr)				
18-24	4 (15%)	3(11%)	3(11%)	10 (12%)
25-35	12 (46%)	13(48%)	13(48%)	38 (48%)
36-55	10 (39%)	11(41%)	11(41%)	32 (40%)
Level of education				
Undergraduate	5 (19%)	6 (22%)	6 (22%)	17 (21%)
Graduate	4 (54%)	14 (52%)	14 (52%)	42 (53%)
Postgraduate	7 (27%)	7 (26%)	7 (26%)	21 (26%)
Marital status				
Married	10(38%)	9 (34%)	9 (34%)	28 (35%)
Unmarried	13 (50%)	14 (52%)	14 (52%)	41 (51%)
Divorced	2 (8%)	2 (7%)	2 (7%)	6 (8%)
Widowed	1 (4%)	2(7%)	2(7%)	5 (6%)

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

Groups	Pretest		Posttest		p-Value ^b
	Mean	SD	Mean	SD	
WL					
State Anxiety	39.87	±5.85	40.16	±6.88	0.805
Trait Anxiety	32.13	±5.16	32.23	±6.49	0.899
EG 1					
State Anxiety	39.50	±5.97	33.66	±6.99	0.001*
Trait Anxiety	32.03	±6.33	27.81	±6.86	0.008*
EG 2					
State Anxiety	39.84	±5.90	35.19	±5.15	0.001*
	0.934		0.003*		
Trait Anxiety	32.44	±5.02	29.03	±6.57	0.024*
p-value ^a	0.942		0.004*		

*p < 0.05

^ap-value is calculated by Kruskal-Wallis test between groups^bp-value is calculated by Wilcoxon test between study phases**Table 4: Cohen's D, Hedges' G and Percentage Change in intervention groups.**

Group	Cohen's D Posttest	Hedges' G Posttest	% Of change Pretest-Posttest
EG1			
State Anxiety	0.93	0.93	-14.78
Trait Anxiety	0.66	0.66	-13.18
EG2			
State Anxiety	0.78	0.78	-11.67
Trait Anxiety	0.49	0.48	-10.51

alternative medicine therapies like aromatherapy³⁰. Nevertheless, this point constitutes one of the limitations because only few participants belonged to least educated population and our study does not show how these therapies can work in a different population, besides, the participant number is insufficient hence results cannot be generalized.

Literature describes that EOs can regulate brain health and functions associated with mood, anxiety and depression³¹. Indeed, evidence in anxiety animal models and some clinical trials show that EOs used in aromatherapy can alleviate anxiety symptoms¹⁰. This data agrees with our findings where participants in EG1 and EG2 show a decrease in anxiety scores after intervention. Although it is true that anxiety levels are still high according to STAI, the decrease in anxiety scores generates a certain effect size denoting the intervention effectivity. In this sense,

state anxiety scores showed a large size effect in EG1, however it was found medium size effect in outcomes belonging to the rest of categories in both experimental groups. This is in accordance with other studies which investigated the effectiveness of EOs in reducing state anxiety³². A meta-analysis indicated that aromatherapy could mitigate especially temporary anxiety¹³. It is also remarkable to affirm that state anxiety is related to temporary situations that changes every moment and when disappear, the individual no longer experience anxiety, but trait anxiety is related to permanent personality features of people³³.

Moreover, significant differences between experimental groups may be due to the fact of difference constitution of the two essential oils tested. In view of this, *L. alba* essential oil shows the major quantitative differences in components with anxiolytic effect in comparison with *L.*

citriodora. Indeed, carvone is considered as a depressor of the central nervous system, interacting with GABA_A receptors in the brain after crossing the blood-brain barrier³⁴. The mechanism is similar to BDZs that exert a positive allosteric modulatory effect increasing inhibitory GABAergic neurotransmission³⁵. Furthermore, the highest amount of linalool was found in *L. alba* compared to *L. citriodora*. The evidence about anxiolytic effect of linalool inhalation is widely reported, although its anxiolytic mechanism of action is not clearly, studies suggest that anxiolytic effects are triggered by olfactory input evoked by linalool odor exposure that activate anxiolytic circuits involving GABAergic transmission via BDZ-responsive GABA_AR³⁶.

Limonene is another component present in both EOs, in this case, a study demonstrate that this component can inhibit anxiety-related behavior through A2A receptor-mediated regulation of DAergic and GABAergic neuronal activity³⁷. Besides it is possible that terpenes such as geraniol and nerol present in *L. citriodora*, also exert anxiolytic action³⁸. However, not only EOs components by themselves may present anxiolytic effects, but there is also evidence that properties of EOs cannot be attributed to a single component because these could be the result of the synergistic effects of its constituents³⁹.

CONCLUSION

According to our investigation, the essential oils extracted from *Lippia alba* and *Lippia citriodora* may be useful as a mean to counteract anxiety, specifically state anxiety showing short-terms benefits.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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GRAPHICAL ABSTRACT



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