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Anxiolytic-like effect of *Lippia alba* essential oil: A randomized, placebo-controlled trial

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ABSTRACT

Aim: This study was conducted to examine whether aromatherapy with essential oil of *Lippia alba* can reduce anxiety levels in a normal population.

Methods: The oil was extracted by hydrodistillation for 3 hours using a modified Clevenger-type apparatus and then chemical composition was investigated by a combination of gas chromatography analysis and gas chromatography-mass spectrometry. Moreover, a randomized, double blind, placebo-controlled trial was conducted, where 62 participants were divided into two groups, comprising a control group treated with placebo, and an experimental group (EG) treated with aromatherapy based on *Lippia alba* essential oil. The anxiety index was evaluated pretest by State-Trait Anxiety Inventory. Measures were taken twice: during pretest and posttest.

Results: The chemical analysis showed that carvone was the main component (62.8%). State and trait anxiety scores showed a decrease in the posttest study phase in comparison with pretest in the EG ($p < 0.005$ for state anxiety and $p < 0.05$ for trait anxiety). Cohen's d score was 1.06 in state anxiety, while it was 0.72 for trait anxiety. The percentages of change showed reductions of anxiety variable ranging between 15.50% for state anxiety and 12.25% for trait anxiety.

Conclusion: These results suggest that aromatherapy based on essential oil of *Lippia alba* may be useful as a means to counteract anxiety.

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Introduction

Nowadays, anxiety is one of the most common and prevalent health problems with high rates of relapse and recurrence [1]. According to the Global Burden of Disease Study, anxiety is one of the main causes of global disability [2], because it is associated with chronic conditions as migraine, chronic respiratory disorders, gastrointestinal problems, heart disease, among others [3]. It also impacts the existential aspects of individuals like employment; indeed, people with anxiety experience lower employment rates, higher absenteeism rates, as well as low productivity [2].

In spite of the success of short-term treatment of anxiety with medication, the rate of improvement on chronic treatment is disappointing. To this is added the fact that anxiolytic drugs produce various side effects with indiscriminate use [4]. In this context, complementary and alternative medicine (CAM) approaches have gained interest. This is due to the fact that CAM therapies are perceived as effective, natural, economical, and with fewer side effects [5]. One of these interventions is aromatherapy, which is based on the use of essential oils (EOs) as the main therapeutic agents. These volatile compounds are a mixture of saturated and unsaturated hydrocarbons, alcohol, aldehydes, esters, ethers, ketones, oxides phenols, and terpenes [6]. They are extracted from various parts of aromatic plants such as the flowers, leaves, stems, fruits, seeds, roots, rhizomes, barks, and resins [7].

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These oils are frequently isolated via steam distillation, hydrodistillation, and cold pressing methods [8]. They are administered via inhalation, massage, compress, and rarely, they are taken internally through gelatin capsules, honey water, vaginal pessaries, and suppositories [9].

Some researchers have found that aromatherapy can reduce anxiety [10], stress [11], and there is even some clinical evidence that EOs can be used as a promising treatment in preoperative anxiety and for reducing perioperative pain [12].

Lippia alba is an aromatic shrub that belongs to the Verbenaceae family. It has a wide distribution, especially in the tropical and subtropical as well as temperate zones of the Americas, Africa, and Asia [13]. *L. alba* is traditionally used to treat headaches, measles, rashes, stomachache, indigestion, diarrhea, pain, and for the nerves. Its essential oil has several chemotypes depending on the geographic location and the characteristics of the soil and climate [14,15]. Different laboratories have determined the antibacterial [16], antifungal [17], anesthetics [18], and anxiolytic [19] effects of *L. alba* EOs.

The present investigation was conducted to examine whether aromatherapy with EOs of *L. alba* can reduce anxiety levels in a normal population.

Materials and Methods

Plant material

The leaves of *Lippia alba* were collected from the Rosa Elena de los Rios Martinez Botanical Garden of Medicinal Plants National University of Trujillo at 34 meters elevation with sandy loam soil, located in Trujillo district, La Libertad Region, Peru. The sample collection was conducted in the months of May to June after the Niño Costero phenomenon in 2017. The voucher specimen was prepared and identified by Eric Frank Rodríguez Rodriguez, PhD, and deposited at the Herbarium Truxillense (HUT) of the National University of Trujillo, under registration number 59148.

Essential oils extraction

The powdered plant material (100 g) of the leaves of *L. alba* and 1,000 ml distilled water were placed in a round-bottomed flask and connected to a Clevenger-type apparatus. Hydrodistillation was completed after 3 hours of boiling. Then the oil was dried over anhydrous sodium sulfate and stored in a refrigerator in amber glass vials at 4 °C for further use in experiments [20].

Determination of essential oil composition

Gas chromatography analysis (GC) was performed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector, using the following conditions: column temperature, 40°C (8 minutes) to 180°C at 3°C/min, 180–230°C at 20°C/min, 230°C (20 minutes); 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 (30 m x 0.25 mm i.d., 0.25 µm film thickness), column temperature, 40°C (8 minutes) to 180°C at 3°C/min, 180–230°C at 20°C/min, 230°C (20 minutes); 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 the literature data and with those in the NIST 2011 mass spectra library as well as Wiley library [17].

Study design and sample

An experimental study with measures at pretest–posttest was conducted. 62 participants were divided into two groups of 31 participants, comprising a control group (CG) treated with placebo (sunflower oil was selected as placebo as it has similar texture and possesses no known therapeutic effect) [21], and an experimental group (EG) treated with aromatherapy based on *Lippia alba* essential oil. These two groups were compared in a randomized, double blind, placebo-controlled trial.

Study procedure

A free aromatherapy course was offered through local press to recruit participants. 79 people were enrolled and 62 took part in this research between July and August 2017. The inclusion criteria included male and female participants between the ages of 18 and 45 and they were required to have a State-Trait Anxiety Inventory (STAI) score of greater than 20 in both the scales; meanwhile the exclusion criteria were participants with previous practice of alternative therapies such as meditation, tai chi or yoga, psychiatric treatment, and pregnancy.

31 participants for each group were randomized by a person not involved in the study by

utilization of a random number table. After CG and EG were formed, a basic questionnaire consisting of social-demographic characteristics was applied in order to characterize the participants. After that, an anxiety self-report instrument was administered (pretest) and filled by all the participants. Two schedules were disposed for each intervention group (one in the morning and one in the afternoon). The *L. alba* essential oil and placebo (sunflower oil) were placed every session in identical amber glass vials marked with the code A and B, respectively. Both participants and researcher did not know the meaning of the codes. A psychotherapy room (4×4 m size) of Integral Psychotherapy Center was used for the experiments. The windows were closed hermetically during stimulus administration and the participants sat in ergonomic chairs forming a circle. Five environmental diffusers were used for administrating oil and placebo by inhalation. These were placed one in each corner of the therapy room and one in the middle of the circle of participants. The essential oil dose required to saturate the experimental room was four drops of 2% essential oil = 0.2 ml [22], and the placebo dose required was also four drops. All the groups had 30-minute intervention sessions from Monday to Saturday for two weeks (12 sessions). After that, an anxiety self-report instrument was administered (posttest) to 55 participants who remained to the end of the study (seven participants, three in EG and four in CG, were lost) (Fig. 1). When the offered course finished, all the participants were informed about the investigation program goals and signed a consent form in which confidentiality and anonymity were guaranteed.

The study was approved by the Ethics and Research Committee of the Postgraduate School of Cesar Vallejo University, as well as this investigation was performed in accordance with the Declaration of Helsinki.

Instruments

Anxiety was evaluated using the 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 the 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 [23,24]. Inventory was validated for the local population in a previous study [22].

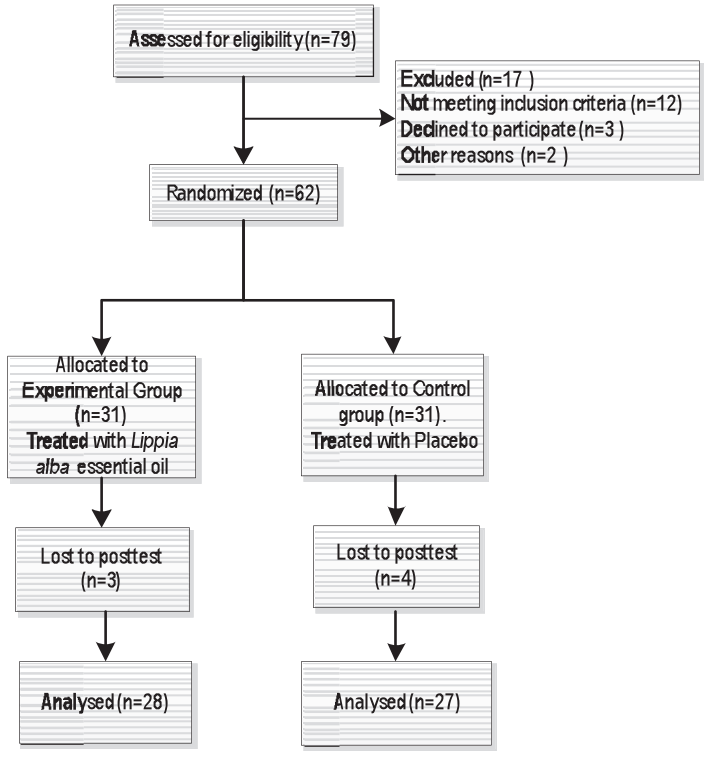

Figure 1. Flowchart of the study.

Table 1. Main chemical constituents (%) of the essential oil of *Lippia alba.*

Composition	RI	%
α-Thujene	922	0.2
α-Pinene	928	0.1
Sabinene	959	2.8
1-octen-3-ol	965	0.5
Myrcene	973	0.3
Limonene	1,027	6.8
Gamma-terpinene	1,042	0.9
Linalool	1,086	1.9
Terpinen-4-ol	1,151	T
Trans-dihydrocarvone	1,178	0.2
Carvone	1,223	62.8
Piperitone	1,241	1.1
Bornyl acetate	1,266	0.1
Thymol	1,285	0.3
Piperitenone	1,310	2.1
Eugenol	1,359	T
α-copaene	1,372	0.4
β-bourbonene	1,387	0.6
β-cubebene	1,394	0.5
β-elemene	1,399	0.5
β-caryophyllene	1,422	0.2
Aromadendrene	1,434	0.6
α- humulene	1,460	T
Germacrene D	1,473	6.1
α-muurolene	1,492	3.2
δ-cadinene	1,514	0.3
Germacrene B	1,559	T
Bulnesol	1,571	1.6
(E)-Nerolidol	1,584	2.4
α-muurolol	1,628	0.2
α-cadinol	1,654	0.1
14-Hydroxy-b-caryophyllene	1,660	0.2
Nd	1,720	0.1
(E)-Phytol	2,084	0.6
Nd	2,230	0.1
Total identified (%)		97.8

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

Data analysis

Data were presented as mean ± standard deviation (SD). Mann–Whitney U test was used to determine significant differences between CG and EG, while Wilcoxon test for paired samples was used to determine significant differences between the study phases. These tests were chosen because data did not conform to the normal distribution. Cohen's D and percentage change were calculated between the pretest and posttest scores. All statistical analysis was performed using SPSS v.23.0 (IBM Corp., Armonk, NY, USA).

Results

Chemical composition of the essential oil of *Lippia alba* is shown in Table 1, where 33 constituents were identified, representing 97.8% (area percent) of the total oil content, among which carvone (62.8%), limonene (6.8%), germacrene D (6.1%), α-muurolene (3.2%), sabinene (2.8%), (E)-nerolidol (2.4%), piperitenone (2.1%), linalool (1.9%), and bulnesol (1.6%) were the major components.

Table 2. Socio-demographic and clinical data of participants in study.

Socio-demographic data	Control group	Experimental group	Total
Gender			
Male	13 (48%)	12 (43%)	25 (46%)
Female	14 (52%)	16 (57%)	30 (54%)
Age(yr)			
18–24	5 (19%)	3 (11%)	8 (15%)
25–35	15 (56%)	16 (57%)	31 (56%)
36–45	7 (25%)	9 (32%)	16 (29%)
Level of education			
High school	2 (7%)	1 (4%)	3 (5%)
Undergraduate	11 (41%)	13 (46%)	24 (44%)
Graduate	9 (33%)	10 (36%)	19 (35%)
Postgraduate	5 (19%)	4 (14%)	9 (16%)
Marital status			
Married	9 (33%)	10 (36%)	19 (34%)
Unmarried	17 (63%)	16 (57%)	33 (60%)
Divorced	0 (0%)	2 (7%)	2 (4%)
Widowed	1 (4%)	0 (0%)	1 (2%)
Anxiety treatment provided			
Psychological	5 (19%)	4 (14%)	9 (16%)
Pharmacological	0 (0%)	0 (0%)	0 (0%)
None	22 (81%)	24 (86%)	46 (84%)

Table 2 presents the socio-demographic and clinical data of the analyzed participants, where 25 were male and 30 were female. CG was formed by 13(48%) males and 14(52%) females, while EG was formed by 12(43%) males and 16(57%) females. The majority of participants were between 25 and 35 years old (n = 31; 56%), followed by the participants between 36 and 45 years old (n = 16; 29%); and the rest between 18 and 24 years old (n = 8; 185%). Three people had (5%) attended high school, 24 (44%) were undergraduate students, 19(35%) were graduates, and nine (16%) were postgraduates. In relation to their marital status, 33 (60%) were unmarried, 19 (34%) were married, two (4%) were divorced, and just one participant was widowed. The majority of participants had never attended to treatment (n = 46; 84%), 9 (16%) attended to psychological treatment, and none had visited a psychiatrist for pharmacological treatment. Finally, both CG and EG were constituted by the same average number of participants by variable.

Table 3 shows the mean score and SDs for anxiety based on STAI, while EG does not present

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

Groups	Pretest		Posttest		*p*-value[b]
	Mean	SD	Mean	SD	
CG					
State anxiety	36.42	±5.64	36.71	±5.58	0.399
Trait anxiety	30.16	±6.32	30.94	±6.96	0.500
EG					
State anxiety	36.65	±6.48	30.97	±5.23	0.005*
p-value[a]	0.766		0.001**		
Trait anxiety	30.13	±7.31	26.44	±5.54	0.046*
p-value[a]	0.676		0.018*		

*$p < 0.05$, **$p < 0.005$

[a]*p*-value is calculated by Mann–Whitney U test between groups

[b]*p*-value is calculated by Wilcoxon test between study phases.

differences in the pretest scores in comparison with CG ($p > 0.05$ for state and trait anxiety); somehow, differences are shown in the posttest scores ($p < 0.005$ for state anxiety and $p < 0.05$ for trait anxiety).

Besides, anxiety scores show a decrease in the posttest study phase (30.97 and 26.44 for state and trait anxiety, respectively) in comparison with pretest (36.65 and 30.13 for state and trait anxiety, respectively) in EG ($p < 0.005$ for state anxiety and $p < 0.05$ for trait anxiety). These results show a change in STAI scores after the intervention. Meanwhile, CG shows a slight increase in the posttest scores in comparison with the pretest scores, but it does not show statistically significant differences to suggest considerable changes in anxiety levels ($p > 0.05$).

Regarding the amount of change in the mean scores at posttest, it is observed that Cohen's *d* score is $d = 1.06$ for state anxiety. Scores over 1 mean a large size effect, which suggests the intervention was effective for improving state anxiety. In the case of trait anxiety, Cohen's *d* score is $d = 0.72$. In this sense, scores over 0.50 and less than 1 indicate a medium size effect, suggesting that aromatherapy was moderately effective in improving trait anxiety. Finally, percentages of change between the pretest and posttest measures show a reduction of 15.50% for state anxiety and 12.25% for trait anxiety. All these results show a decrease in the anxiety scores (Table 4).

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

Group	Cohen's d posttest	% of change pretest-posttest
EG		
State anxiety	1.06	−15.50
Trait anxiety	0.72	−12.25

Discussion

The chemical analysis of *L. alba* essential oil showed that carvone is the main component and could be classified as carvone chemotype. This is in agreement with data from another study where this chemotype for Peru is also reported [25].

In relation to the socio-demographic and clinical data of participants, the majority were young adults and adults with university studies. This is in agreement with a previous study conducted by our research team, where participants were demographically similar [26]. This point constitutes one of the limitations, because there were no participants that represented low educational levels and our study does not show how these therapies can work in a different population. To this is added the fact that the participant number may not be enough to generalize the results. Another limitation is that due to homogeneity sample, correlations between socio-demographic and clinical data with anxiety scores are not displayed. It is appropriate to remark that in the Peruvian context, the main provider of CAM therapies is Essalud, a type of national health insurance, which covers only the salaried population. Their services are not well known by the general population, nor can the low income population afford it [27]. In fact, some researchers state that most educated sectors are more interested in CAM therapies [28].

There is some clinical evidence in favor of the EO use to reduce preoperative anxiety [29], as well as anxiety in dental treatments [30]. Indeed, findings confirm that aromatherapy can reduce anxiety levels [31]. These data are in agreement with our findings where participants show a decrease in the anxiety scores after intervention. Although more precisely speaking, state anxiety scores showed a large size effect in comparison with trait anxiety

scores that only exhibited medium size effect. This is in accordance with other studies which investigated the effectiveness of aromatherapy in reducing a state anxiety situation [11, 32]. This may be due to the fact that state anxiety is related to temporary situations that change every moment, and when they disappear, the person no longer experiences anxiety; on the other hand, trait anxiety is related to particular and permanent personality features [33]. Nevertheless, further investigations are needed to determine if the essential oil of *Lippia alba* can be useful in pathological anxiety.

Besides, other studies found that *L. alba* essential oil (carvone chemotype) show anxiolytic effect in rats, pointing that carvone could be responsible for its action as a tranquilizer, due to its effect as a depressor of the central nervous system, interacting with $GABA_A$ receptors in the brain after crossing the blood–brain barrier [19]. Therefore, carvone attenuate neurons in limbic and septo-hippocampal systems, structures involved in suppressing the response of "active-avoidance" [34]. Other components related to exert an anxiolytic effect similar to that seen with diazepam are limonene and linalool [35], also present in the *L. alba* essential oil analyzed in this study. In the case of limonene, a study suggests a non-benzodiazepine mechanism [36]. Meanwhile linalool has antagonistic action on glutamatergic receptors such as N-methyl-D-aspartate receptors, which may explain its sedative effect [37].

Conclusion

These results suggest that aromatherapy based on essential oil of *Lippia alba* may be useful as a means to counteract anxiety.

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