Anxiolytic-like Effect of Luma chequen Essential Oil: A Pilot Study

Paul Alan Arkin Alvarado-García1,*, Marilú Roxana Soto-Vásquez2, Luis Enrique Rosales-Cerquin3, Demetrio Rafael Jara-Aguilar2, Santiago M. Benites4

ABSTRACT
Introduction: Essential oils are complex substances used widely extended in the form of aromatherapy or phytotherapy, some of them as agents to relieve anxiety and stress. In this sense this study was conducted to examine the anxiolytic-like effect of Luma chequen essential oil. Methods: The essential oil was extracted by steam distillation and the chemical composition was investigated by Gas chromatography with flame-ionization detection (GC-FID) and Gas chromatography-mass spectrometry (GC–MS). Moreover, a randomized, double blind, placebo-controlled trial was conducted, where 48 participants were divided into two groups, comprising a control group (CG) treated with placebo, and an experimental group (EG) treated with aromatherapy based on Luma chequen 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 α-pinene was the main component (56.5%). State and Trait anxiety scores showed a decrease in posttest study phase in comparison with pretest in experimental group compared to placebo (p<0.005 for state anxiety and p<0.05 for trait anxiety). Cohen’s d score was 0.84 in State anxiety, while it was 0.52 for Trait anxiety. Percentages of change showed reductions of anxiety variable ranging between 14.94% for State anxiety and 13.60% for Trait anxiety. Conclusions: These results suggest that aromatherapy based on essential oil of Luma chequen was moderately effective in improving anxiety.

Key words: Essential oil, Anxiety, Luma chequen.

INTRODUCTION
Different studies have consistently shown that anxiety is among the most prevalent health problems in the 21st century and it is one of the most prevalent psychological problems during the COVID-19 pandemic. The WHO Global Burden of Disease Study estimates that anxiety is one of the main causes of world disability, because it is associated with chronic conditions such as migraine, gastrointestinal problems, heart disease, etc. It also negatively impacts the existential aspects of individuals such as work performance and social interactions.

Pharmacological treatment usually employs benzodiazepines (BDZs) and antidepressants, nevertheless, BDZs produce side effects as lethargy, retrograde amnesia, drowsiness, dizziness, and vertigo, in addition, antidepressants can provoke weight gain, tachycardia, sexual dysfunction among others, affecting clinical adherence. To this is added the fact that synthetic drugs produce other important drawbacks such as indiscriminate use, tolerance, and dependence.

In this context, non-pharmaceutical treatment has received considerable attention. In fact, investigations in herbal medicine have revealed a variety of medicinal plants may provide benefits in anxiety treatment, highlighting the use of essential oils (EOs) as a useful alternative therapy to relieve anxiety.

EOs have been used traditionally from ancient times, these metabolites are usually highly complex mixtures of natural compounds, both polar and non-polar, and are defined by The International Organization for Standardization (ISO) (ISO/ D189235.2), as products made by distillation with either water or steam or by mechanical processing or by dry distillation. EOs are obtained from different parts of aromatic plants, although at present new sources of these secondary metabolites are examined, such as food and vegetal wastes.

Some researchers have found that aroma inhalation reduces anxiety. In fact, Lavandula angustifolia (lavender), Rosa damascena (rose), Citrus sinensis (orange), Citrus bergamia (bergamot), Salvia sclarea (clary sage), Matricaria recutita (Chamomile) and Pelargonium species EOs can lead to decrease levels of anxiety.

In the clinical context, a study confirmed the effectiveness of geranium EOs to manage anxiety of nulliparous women during labor. Likewise, an investigation found that aromatherapy is a cost-effective method that can reduce anxiety in hospitalized patients with acute coronary syndrome, as well as in preoperative anxiety.

Luma chequen is a species of flowering shrub in the family Myrtaceae, growing between 2500 to 4000 m elevation, native to the South American Andes between Perú, Bolivia, Chile, and Argentina. Its leaves and twigs are traditionally used for the treatment of gastrointestinal and respiratory disorders, post-parturition infections, insomnia and anxiety. Luma chequen essential oil is marketed and use in Peru for calming and balancing emotions due to their components such as linalool, limonene among others with anxiolytic properties. Thus, the present investigation was conducted to evaluate whether aromatherapy based on Luma chequen essential oil can reduce anxiety levels.

MATERIAL AND METHODS

Plant material

The fresh leaves of *Luma chequen* were collected from Yungay village at 2,458 meters elevation, located in Yungay district, Ancash Region, Peru. The sample collection was conducted in the month of August 2019. 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. Subsequently leaves were milled, and the powdered plant material (100 g) and 1000 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. Finally, 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.

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 two fused silica capillary columns with two different stationary phases (SPB-1 and SupelcoWax 10, 30 m x 0.2 mm, 0.20 μm). GC was performed in a Hewlett Packard 6890 gas chromatograph with a flame ionization detector (FID), using the following conditions: oven temperature programmed at 70°-220°C (3°C/min), 220°C (15 min); injector temperature: 250°C; detector temperature: 250°C; split ratio 1:40; carrier gas, helium. GC-MS was carried out using a Hewlett-Packard 6890 series gas chromatograph coupled with a mass selective detector Hewlett Packard MSD 5972, using the same analytical conditions as above; interface temperature: 250°C; MS source temperature: 230°C; EI mode 70 eV. Finally, the identification of essential oil constituents was based on comparing their GC retention indices (RI) and mass spectra with literature data and with those in the NIST 2011 mass spectra library as well as Wiley library.

Study design and sample

An experimental study with measures at pretest-posttest was conducted. 48 participants were divided into two groups of 24 participants, comprising a control group (CG) treated with placebo, which was a non-essential oil-based scented shampoo, and an experimental group (EG) treated with aromatherapy based on *L. chequen* essential oil.

Study procedure

A free aromatherapy course was offered through social media to recruit participants. 61 people were enrolled and 48 took part in this investigation between November 2019 and December 2019. Inclusion criteria included male and female participants between the ages of 18-45 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 treatment, and pregnancy. 24 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 consisted of social-demographic characteristics was applied to characterize participants. After that, an anxiety self-report instrument was administered (pretest) and filled by all participants. Two schedules were disposed for each intervention group (one in the morning and one in the afternoon) to prevent them from influencing each other. The *L. chequen* essential oil and placebo were placed every session in identical amber glass vials marked with the code A and B, respectively. It was used as placebo a commercial shampoo (Johnson’s baby shampoo, free from sulfates and parabens). Both, participants, and researchers did not know of the code meaning. In addition, one researcher oversaw the experimental group, and another was in charge of the control group. Both were held incommunicado throughout the experiment to minimize bias; besides, they wore face masks (3M1810N95), according to the methodology used by Ndao et al.

A Psychotherapy room (4x4 m size) of Integral Psychotherapy Center was used for experiments. Windows were closed hermetically during stimulus administration and participants sat in ergonomic chairs forming a circle. Five essential oil diffusers with 200 mL capacity were used for administrating oil and placebo by inhalation. These were placed one in each corner of therapy room and one in the middle of the circle of participants. The essential oil dose required to saturate the experimental room was 4 drops of 2% essential oil=0.2 mL; and placebo dose required was also 4 drops. All groups had 30 minutes intervention sessions from Monday to Saturday for two weeks (12 sessions). Next, an anxiety self-report instrument was administered (posttest) to the 44 participants who remained to the end of the study (four participants, one in EG and three in CG, were lost) (Fig. 1). 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). 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. Validations and reliability coefficients for local population were found in a previous study Inventory was validated for local population in a previous study.

Data Analysis

Data were presented as mean ± standard deviation (SD). Independent samples t Test was used to determine significant differences between CG and EG, while Paired Samples t Test was used to determine significant differences between the study phases. These tests were chosen because data conformed to the normal distribution. Cohen’s D 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

Chemical composition of the essential oil of *Luma chequen* is shown in Table 1, where 31 components were identified, representing 96.1% (area percent) of total oil content, among which α-pinene (56.5%), 1,8-cineole (8.5%), linalool (6.8%), β-pinene (6.4%), isobutyl isobutyrate (1.9%), α-Terpineol (1.8%), p-cymene (1.2%) and camphene (1.1%) were the major constituents.

Table 2 presents socio-demographic and clinical data of analyzed participants, where 19 were male and 23 were female. CG was formed by 9(43%) male and 12(57%) female, while EG was formed by 10(44%) male and 13(56%) female. Most participants were between 25-35 years.
Table 1: Main chemical constituents (%) of the essential oils of Luma chequen.

<table>
<thead>
<tr>
<th>Components*</th>
<th>RI</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isobutyl isobutyrate</td>
<td>924</td>
<td>1.9</td>
</tr>
<tr>
<td>α-Thujene</td>
<td>927</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Pinen</td>
<td>932</td>
<td>56.5</td>
</tr>
<tr>
<td>Camphene</td>
<td>945</td>
<td>1.1</td>
</tr>
<tr>
<td>β-Pinen</td>
<td>968</td>
<td>6.4</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>994</td>
<td>1.2</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1018</td>
<td>8.5(^*)</td>
</tr>
<tr>
<td>Limonene</td>
<td>1025</td>
<td>5.8(^*)</td>
</tr>
<tr>
<td>cis-Linalool oxide</td>
<td>1066</td>
<td>0.1</td>
</tr>
<tr>
<td>Linalool</td>
<td>1084</td>
<td>6.8</td>
</tr>
<tr>
<td>Fenchol</td>
<td>1101</td>
<td>0.2</td>
</tr>
<tr>
<td>Campholenal</td>
<td>1115</td>
<td>0.1</td>
</tr>
<tr>
<td>Pinocarveol</td>
<td>1120</td>
<td>0.2</td>
</tr>
<tr>
<td>cis-Verbenol</td>
<td>1129</td>
<td>0.6</td>
</tr>
<tr>
<td>Pinocarvone</td>
<td>1136</td>
<td>0.1</td>
</tr>
<tr>
<td>bornol</td>
<td>1148</td>
<td>0.3</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1160</td>
<td>0.3</td>
</tr>
<tr>
<td>Myrtenal</td>
<td>1171</td>
<td>0.1</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>1182</td>
<td>1.8</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1190</td>
<td>2.0</td>
</tr>
<tr>
<td>Aromadendren</td>
<td>1432</td>
<td>0.3</td>
</tr>
<tr>
<td>Allo-aromadendrene</td>
<td>1451</td>
<td>0.4</td>
</tr>
<tr>
<td>β-Selinene</td>
<td>1476</td>
<td>0.8</td>
</tr>
<tr>
<td>α-Muurolene</td>
<td>1494</td>
<td>0.4</td>
</tr>
<tr>
<td>δ-Cadinene</td>
<td>1510</td>
<td>0.3</td>
</tr>
<tr>
<td>Nd</td>
<td>1559</td>
<td>0.2</td>
</tr>
<tr>
<td>Globulol</td>
<td>1571</td>
<td>0.9</td>
</tr>
<tr>
<td>Total identified (%)</td>
<td>96.1</td>
<td></td>
</tr>
</tbody>
</table>

RI, Retention index; t= traces (<0.1%); –, not detected.

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

<table>
<thead>
<tr>
<th>Socio-demographic data</th>
<th>CG</th>
<th>EG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-24</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>25-35</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>36-45</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Level of education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Undergraduate</td>
<td>9</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Graduate</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Postgraduate</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Unmarried</td>
<td>10</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Divorced</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Widowed</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Anxiety treatment provided</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Pharmacological</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>16</td>
<td>17</td>
<td>33</td>
</tr>
</tbody>
</table>

old (n=30; 68%), followed by the participants between 18-24 years old (n=9; 21%); and the rest between 36-45 years old (n=5; 11%). 3 people (7%) attended to high school, 19 (43%) were undergraduate students, 15 (34%) were graduated and only 7 (16%) were postgraduate. In relation to their marital status, 19 (43%) were unmarried, 18 (41%) were married, 3 (11%) were divorced and 2 participants were widowed. Majority of participants never attended a treatment (n=33; 75%), 11 (25%) attended a psychological treatment and no one attended a psychiatrist for pharmacological treatment. Finally, both CG and EG were constituted by same average number of participants by variable.

Table 3 shows the mean score and SDs for anxiety based on STAI, where EG does not present differences in pretest scores in comparison with CG (p>0.05 for state and trait anxiety); however, 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 (32.17 and 28.42 for State and Trait anxiety respectively) in comparison with pretest (37.82 and 32.79 for State and Trait anxiety respectively) in experimental group (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 show a slight increase in posttest scores in comparison with pretest scores, but it does not show statistically significant differences (p>0.05).

Regarding the amount of change in the mean scores at posttest, it is observed that all Cohen’s d scores are over 0.50 and less than 1 (d=0.84 for state anxiety and d=0.52 for trait anxiety). These scores indicate a medium size effect, suggesting that aromatherapy was moderately effective in improving anxiety. Finally, percentages of change between pretest and posttest measures show reduction of 14.94% for State anxiety and 13.60% for Trait anxiety. All these results show a decrease in anxiety scores (Table 4).

DISCUSSION

The chemical analysis of L. chequen essential oil showed that α-pinene, 1,8-cineole, linalool and β-pinene are the main components. This agrees with data from other investigations where these are also the major constituents; however, another research study found D-limonene, eucalyptol and α-pinene as the main components, but this last one presented lower values in comparison with our investigation. This may be due to the difference of collection time, in our case and the rest of investigations with the same results, collection was done in spring while in the latter research study collection was done in fall. In fact, content of monoterpenes is more abundant in spring than in fall. Although studies that involve the geographical variation are needed, because Andean countries like ours have different altitudinal floors.

Regarding socio-demographic and clinical data of participants, the majority were young adults and adults with university studies as well with no pharmacological treatment. This is in concordance with a previous study conducted by our research team, where participants were demographically similar. This point constitutes one of the limitations because only few participants belonged to the least educated group. This may be due to the difference of collection time, in our case and the rest of investigations with the same results, collection was done in spring while in the latter research study collection was done in fall. Regarding the amount of change in the mean scores at posttest, it is observed that all Cohen’s d scores are over 0.50 and less than 1 (d=0.84 for state anxiety and d=0.52 for trait anxiety). These scores indicate a medium size effect, suggesting that aromatherapy was moderately effective in improving anxiety. Finally, percentages of change between pretest and posttest measures show reduction of 14.94% for State anxiety and 13.60% for Trait anxiety. All these results show a decrease in anxiety scores (Table 4).

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Clinical evidence affirms that EOs can help lower preoperative anxiety, dental patient anxiety, as well as anxiety status during surgery.
Table 3: Group differences of anxiety variable according to State-Trait Anxiety Inventory (STAI).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pretest Mean (SD)</th>
<th>Posttest Mean (SD)</th>
<th>p-Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State Anxiety</td>
<td>37.82 ±6.21</td>
<td>32.17 ±6.05</td>
<td>0.001*</td>
</tr>
<tr>
<td>Trait Anxiety</td>
<td>32.79 ±6.49</td>
<td>28.42 ±7.21</td>
<td>0.042*</td>
</tr>
<tr>
<td><strong>CG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State Anxiety</td>
<td>37.63 ±6.61</td>
<td>37.71 ±7.14</td>
<td>0.956</td>
</tr>
<tr>
<td>Trait Anxiety</td>
<td>32.33 ±6.53</td>
<td>32.50 ±7.05</td>
<td>0.934</td>
</tr>
</tbody>
</table>

*p<0.05

*p-value is calculated by Independent samples t Test between groups

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Cohen's d Postest</th>
<th>% of change Pretest-Postest</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EG</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State Anxiety</td>
<td>0.84</td>
<td>-14.94</td>
</tr>
<tr>
<td>Trait Anxiety</td>
<td>0.52</td>
<td>-13.60</td>
</tr>
</tbody>
</table>

Figure 1: Flowchart of the study.

Aromatherapy based on essential oil of *Luma chequen*, may be considered an alternative treatment option to counteract mild and moderate anxiety states.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

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**CONCLUSION**

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REFERENCES


**CONCLUSION**

Aromatherapy based on essential oil of *Luma chequen*, may be considered an alternative treatment option to counteract mild and moderate anxiety states.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.


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