Original Article

Effect of Citrus aurantium essential oil on anxiety in nursing students during exams

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Abstract: Anxiety is an emotional state characterized by feelings of apprehension, insecurity, and fear, and is a common reaction to stressful situations. This condition is often observed among nursing students, especially during exam periods, and can compromise academic performance. Given this, complementary therapies, such as the use of essential oils, have been explored for their possible anxiolytic effects. Citrus aurantium essential oil has stood outforits calming properties, making it a promising alternative for managing anxiety in academic settings. OBJECTIVE: to investigate the anxiolytic potential of Citrus aurantium (bitter orange) essential oil in reducing anxiety levels in nursing students during exam periods. MATERIALS AND METHODS: the research was conducted as double-blind randomized clinical trial. Participants were divided into two groups: one group used Citrus aurantium essential oil by inhalation, while the other used vegetable oil as a placebo. Anxiety was measured after the exams using the Zung Self-Rating Anxiety Scale (SAS). RESULTS: the sample consisted of 42 nursing students, aged between 19 and 53 years, with 50% of the sample concentrated in the 21 to 23 age group. Most participants were female. Regarding anxiety history, they reported anxiety, but many reported that they had no previous diagnosis. Although the experimental group had a slightly higher mean score than the placebo group (53.63 vs. 49.82), there was no statistically significant difference (p=0.273). CONCLUSION: the study indicated that Citrus aurantium essential oil did not reduce anxiety in nursing students during exam periods.

Keywords: Anxiety; Students; Aromatherapy; Citrus aurantium.

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1. Introduction

Anxiety is a natural human response that activates physical and psychological resources and triggers defense reactions to deal with situations that pose threats or challenges. It acts as a warning sign for imminent dangers, helping individuals take the necessary measures to face them [1]. However, anxiety becomes pathological when the suffering it causes results in harm to the person, presenting itself in an excessive manner, disproportionate to what causes it or qualitatively different from what is considered typical, affecting quality of life, emotional well-being, or daily performance [2].

According to information from the World Health Organization (WHO), Brazil is the country with the highest number of anxious people in the world, with 9.3% of the population, which corresponds to approximately 18.6 million Brazilians at this time [3]. Among this population, nursing students stand out, as they have a higher incidence of anxiety compared to the general population, due to the academic period being a significant source of stress and anxiety. These symptoms can affect students' quality of life, creating a direct link between academic stress and the emergence of emotional problems [4].

The prevalence of anxiety symptoms, especially in nursing students, is not yet well defined, but it is believed that nursing degrees, compared to other degrees, require

students to be more assertive in performing procedures, skilled in patient management, and concerned about not making mistakes, whether in hospital routines or during exams [5]. The pharmacological treatment traditionally used for anxiety disorders often results in a variety of side effects, such as a drop in blood pressure, irregular breathing and heart rate, as well as causing drowsiness, nausea, vomiting, and, in some cases, allergic reactions. In addition, these drugs can lead to physical and psychological complications, including the risk of dependence on psychoactive substances [6].

Therefore, it is increasingly important to conduct scientific studies focused on complementary and integrative therapies, emphasizing the importance of a holistic approach to health care [7]. Such research is essential to validate the effectiveness of these approaches and integrate them more solidly into clinical practice, ensuring that individuals can benefit from evidence-based treatments.

Research conducted by Braga and Domingos has shown significant evidence that aromatherapy is becoming established in the field of health, with growing acceptance and application [8].

This practice is expanding, especially in the field of psychiatry. It is recognized for its cost-effectiveness, both for professionals and patients. Some essential oils have shown positive therapeutic potential in reducing neuropsychiatric and anxiety disorders. We highlight Citrus aurantium, popularly known as bitter orange, whose commercial name is petitgrain, which has become known for its calming and anxiolytic properties and is widely used in aromatherapy to promote relaxation and reduce anxiety symptoms [9]. However, despite its many benefits, there are still few studies exploring its therapeutic potential. The species Citrus aurantium L., commonly referred to as bitter orange, stands out in the scientific and traditional scene for its remarkable phytochemical richness. The diversity of bioactive compounds present in its different parts—such as leaves, flowers, and bark—and the influence of extraction methods give it a very complex and multifaceted pharmacological profile. In the field of essential oils, the central focus of this research, Citrus aurantium has a composition dominated by monoterpenes and other volatiles. Scientific evidence shows that d-limonene emerges as the major component in the peel [10].

Maksoud et al. show that inhaling bitter orange essential oil can help lower levels of cortisol, the stress hormone, and improve mood in situations of anxiety [11].

That said, this study aimed to investigate the anxiolytic role of this essential oil, with the purpose of offering nursing students an accessible, low-cost alternative that has proven to be safe and effective in self-care and anxiety management, especially in pre-test periods, through the use of aromatherapy, encouraging a more holistic and humanized approach within the health field

2. Materials and Methods

2.1. Type of study

This study was conducted as a double-blind, randomized, controlled clinical trial. According to Friedman, Furberg, and DeMets (2010), randomized clinical trials are considered the gold standard for evaluating the effectiveness of medical interventions, as they involve the random distribution of participants among groups, the use of blinding to avoid bias, and strict control of variables, ensuring greater internal validity of the results [12].

2.2. Ethical and legal aspects

The study was approved by the Ethics Committee, under opinion No. 7,477,756.

2.3. Study participants

The study population consisted of 265 students regularly enrolled in the Nursing course. Sixty-two students were invited to participate in the clinical trial through a public invitation made in classrooms. Of these, one individual was excluded due to a history of seizures, two reported headaches during the tests and were withdrawn from the study, one withdrew from the experiment, and eighteen did not attend the olfactory skin sensitivity tests. Thus, the final sample consisted of 42 students who met the inclusion criteria and effectively participated in the study. The students were divided into two groups (intervention and control) in a randomized manner. Randomization ensured that participants were allocated impartially between groups, and placebo control allowed the real effects of treatment to be distinguished from psychological effects or other external factors.

The division of the groups and administration of the oil were double-blind to avoid the influence of the expectations of both the participants and the researcher on the study results [13-14]. Thus, during the study, neither the participants nor the student responsible for conducting the research knew who used the placebo or the essential oil. Neither the participants nor the student responsible for conducting the research knew who used the placebo or the essential oil.

Students who were interested in participating in the study had to attend an appointment with an aromatherapist before the pre-test began, during which skin sensitivity and olfactory tests were performed to assess any contraindications for the use of essential oil. This data was recorded on an aromatherapy assessment form (Appendix 1). This data collection instrument was designed to investigate various aspects related to university students. Initially, it seeks to understand the sociodemographic profile of the participants, providing an overview of the characteristics of the group studied. In addition, the questionnaire addresses the students' previous experiences with the use of essential oils, identifying their familiarity and degree of use of these resources. Another important focus of this study is the experience of anxiety in the academic context, exploring how students perceive and face this challenge in their educational environment. Finally, the instrument also investigates the habits and routines of the participants in order to understand factors that may be related to anxiety management and the general well-being of students.

2.4. Materials

The essential oil used in this study was obtained specifically from the leaves of the *Citrus aurantium* plant, popularly known as bitter orange. This input is supplied and marketed by Laszlo Aromas e Sabores Com. de Alimentos LTDA. According to the manufacturer's specifications, the oil originates from Paraguay and was extracted using the steam distillation method. The raw material used comes from conventional agriculture.

The essential oil was administered at a concentration of 100% in the palm of the hand, with 2 drops (approximately 0.1 mL) applied for inhalation to each participant. The placebo group received rosehip oil at a concentration of 100%, administered in the same way and quantity as the essential oil being tested. The rosehip vegetable oil was produced by the company Vegetal Care, and its liquid has low viscosity and an orange to red color. The inhalation route was chosen for its practicality, rapid absorption, and, above all, its potential to induce relaxation and reduce anxiety. This route is widely used by the population precisely because it allows the volatile compounds in the essential oil to reach the central nervous system effectively, without the side effects often associated with traditional anxiolytics [15].

Participants were properly instructed on the procedure for inhaling *Citrus aurantium* essential oil and the placebo. This instruction was provided both in person during the aromatherapy interview and through a recorded instructional video, which was sent individually to each participant to ensure correct inhalation and standardization of the procedure. The bottles containing *Citrus aurantium* essential oil and the placebo (rosehip oil) were identical, both amber in color, ensuring the masking of the study and maintaining the double-blind nature. Inhalation was performed for 5 minutes, within a period of up to 20 minutes prior to the tests.

2.5. Measure for anxiety assessment

To assess the participants' anxiety levels, we used the Zung Anxiety Scale (Self-Rating Anxiety Scale – SAS). The Zung Self-Rating Anxiety Scale (Zung SAS), designed by William W. K. Zung in 1971, is a self-report instrument frequently used to assess anxiety levels. It consists of 20 items that seek to measure the frequency with which the individual has experienced various anxiety symptoms in a recent period, usually in the last one or two weeks. These items cover cognitive, autonomic, motor, and central nervous system manifestations and are scored on a four-point Likert scale.

This is a standardized, self-administered instrument that covers a wide range of physical and psychological symptoms often associated with anxiety. Assessment of anxiety levels. Among the symptoms assessed are tension, tremors, fear, panic, mental disorganization, difficulty concentrating, insomnia, palpitations, dizziness, tingling in the extremities, and the feeling that something bad is going to happen. Each of these items is scored on a four-point Likert scale, ranging from "rarely or never" to "almost always or always."

The total score on the Zung Anxiety Scale can range from 20 to 80 points, with scores above 40 considered indicative of clinically relevant anxiety symptoms. We chose the Zung Scale because of its simplicity, speed of application, and, above all, its validation for the Brazilian context, which ensures the reliability of the results obtained in our population [16-17].

3. RESULTS

3.1. Sample characterization

The study sample consisted of a total of 42 participants. The mean age of the group was 24.9 ± 6.6 years, indicating a predominantly young adult age group. Regarding gender, the sample showed a clear female predominance, with 95.2% (40 people), while males represented 4.8% (two people).

In terms of occupational status, most participants were students (61.9% - 26 people). Professions not directly related to the study included Nursing Technician (14.3% - 6 people), Administrative Assistant (4.8% - 2 people), and others such as Civil Servant, Telemarketing, Self-employed, Psychologist, Salesperson, Administrative Agent, Teacher, and Intern, each representing 2.4% (one person). Regarding ethnicity, most participants self-identified as mixed race (50.0% - 21 people), followed by white (38.1% - 16 people), with white (9.5% - 4 people) and black (2.4% - 1 person) in smaller proportions. Regarding marital status, the vast majority of the sample was single (73.8% - 31 people).

The remaining participants were married (14.3% - 6 people), single (4.8% - 2 people), or divorced (3.6% - 1 person). Two people (2.4%) answered "YES" and one (1.2%) answered "NO" for marital status, which was interpreted as responses not categorized in the standard options.

Detailed analysis of the participants' health profile revealed that 20 individuals, corresponding to 47.6% of the total sample, were using some type of medication at the time of the study. In contrast, 22 participants (52.4%) were not using medication. It is essential to note that, among those who reported using medication, 18 individuals (42.9% of the total sample) specifically reported using psychotropic medication, drugs that act directly on the central nervous system. This category of medications includes: anxiolytics/benzodiazepines (such as Anxiolytic, Clonazepam, and Alprazolam), antidepressants (such as Venlafaxine, Sertraline Hydrochloride, Desvenlafaxine Succinate, and Fluoxetine), and sleep/hormone regulators (such as Melatonin). Other medications used by participants, not classified as psychotropic, included hormonal contraceptives (Contraceptive, Drospirenone, Mirena IUD, Pill), medications for respiratory diseases/allergies (Alenia, Busonid, Soiriva), and others such as Amora, Jume, Ommy, and Ofetus (whose precise pharmacological classification could not be confirmed).

Additionally, 14 participants (33.3% of the total sample) were undergoing psychotherapy at the time of the study. It should also be noted that 6 participants (14.3% of the total sample) were using psychotropic medication and undergoing psychotherapy at the same time. The inclusion of participants undergoing these treatments was maintained in the study design to reflect the heterogeneity of the student population seeking strategies to manage anxiety. However, this characteristic of the sample is recognized as a point to be discussed in the limitations of the study, as it may have influenced the results and require further analysis to isolate the effect of the intervention.

Most participants reported experiencing anxiety in the university environment, with only 6 participants (14.3% of the total sample) reporting no anxiety at university. The coping strategies adopted by the students were diverse and, although qualitative, allowed us to identify relevant patterns. The most frequently mentioned coping strategies included: seeking psychological help (4 mentions), expressing anxiety before exams (2 mentions), physical reactions such as vomiting and the need to go home (2 mentions each), and managing anxiety through compulsive eating (2 mentions).

Other strategies identified (with 2 mentions each) were: relieving anxiety by crying, public speaking as a trigger/coping mechanism, seeking to calm down through breathing, crying (in general), talking to friends/colleagues/spouse, anxiety due to internship demands, anxiety during exam week or exams in general, therapy (in general), going to the bathroom to try to calm down, practicing relaxation techniques (including breathing exercises), watching movies/series or listening to music, reducing anxiety with physical activity, dealing with anxiety attacks and seeking calm, taking the focus off college to calm down, relaxing by eating a lot, and using medication (sedatives/antidepressants) as a form of treatment.

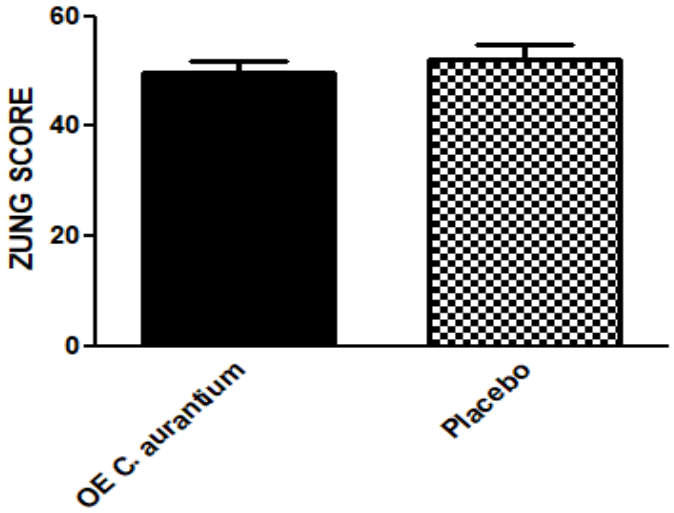
The characterization of the sample revealed important aspects of health and lifestyle. It was observed that none of the participants were smokers, which may be a relevant factor when considering the group's pulmonary and cardiovascular health. A family history of cancer was identified in only two individuals, both specifically breast cancer, indicating a low prevalence of direct genetic risk for this condition. Regarding chronic comorbidities, two participants reported a diagnosis of hypertension, and none had a diagnosis of diabetes. Previous surgical procedures were verified in five participants, with appendectomies and gallbladder removal being the most common procedures. Allergic issues were also reported by about 33% of participants, with the main manifestations associated with medications, food, or dust. Physical activity stood out as a common and positive habit in the sample, with 81% of individuals reporting regular engagement in exercise, predominantly weight training.

Regarding experience with anxiety, all participants confirmed having experienced episodes related to university life**.** The main triggers for these episodes were identified as exams, public presentations, and academic overload. Coping strategies for anxiety were varied, ranging from crying and seeking psychological support to the use of medication and the application of specific breathing and relaxation techniques.

3.2. Effect of the essential oil on students' anxiety during exams

The purpose of this study was to evaluate whether inhaling *Citrus aurantium* essential oil would have an anxiolytic effect on university students, as measured by the Zung Anxiety Inventory (ZUNG), in groups that used the essential oil compared to a placebo. It was observed that there was no significant difference between the groups, since the scores remained close (oil: 49.73 versus placebo: 52.06), with no statistically significant difference. Thus, the results suggest that, under the conditions of this study, the use of *C. aurantium* by direct inhalation was not effective in reducing anxiety levels when compared to placebo.

Figure 1. Effect of *Citrus aurantium* essential oil on anxiety in nursing students during exams using the Zung scale.



4. DISCUSSION AND CONCLUSION

This study sought to investigate the anxiolytic potential of Citrus aurantium essential oil administered by inhalation in university students. However, the results obtained did not demonstrate a significant effect on reducing participants' anxiety levels, as assessed by the Zung Anxiety Scale. This finding diverges from part of the existing literature that suggests anxiolytic properties for Citrus aurantium and other essential oils applied by this route. Previous studies and reviews have pointed to the potential of essential oils, including components of Citrus aurantium (such as linalool and linalyl acetate present in the leaves), to modulate the central nervous system and promote relaxing or anxiolytic effects. The inhalation route, in particular, is often highlighted for its practicality and rapid absorption, allowing volatile compounds to reach the limbic system and other brain areas related to mood and emotions [18-19].

The discrepancy between the results of this study and previously established evidence may stem from a number of methodological factors or particularities of the sample investigated. A substantial limitation identified was the non-compliance of many participants with the stipulated 5-minute inhalation time for the essential oil**.** This premature interruption of exposure may have compromised the delivery of an effective therapeutic dose of bioactive compounds, preventing the desired effects from manifesting. Other variables that may have contributed to the absence of a noticeable effect include the specific chemotyping of the oil used (whose composition may vary depending on the origin and extraction method), the intensity or form of aromatic exposure, or even the environmental conditions at the time of inhalation. The essential oil was applied manually to the palm of the hand, a route susceptible to early volatilization and difficult to standardize, which may have reduced the validity of the findings [20].

The Zung Anxiety Scale (SAS), although validated, may not have been sensitive enough to detect acute changes induced by brief intervention. Other state-anxiety scales could offer better sensitivity. However, physiotherapy can also be an important approach in treating primary dysmenorrhea symptoms. Ortiz et al. [9] demonstrated improvements in primary dysmenorrhea with the use of an exercise program including stretching, specific pelvic strengthening, and relaxation associated with running as a physiotherapy approach. Similarly, Machado et al. [32] used Transcutaneous Electrical Nerve Stimulation (TENS) and microwave diathermy, both of which showed benefits in pain reduction in this population. Moreover, considering a new technique increasingly studied in physiotherapy, Celenay et al. [33] indicated that the use of kinesiotaping may be effective in reducing symptoms such as pain and anxiety in primary dysmenorrhea.

Additionally, the heterogeneity of our sample, which included individuals already undergoing psychotropic drug treatment or psychotherapeutic follow-up, may have introduced biases, making it difficult to attribute any effects exclusively to the essential oil. Although it did not achieve the expected outcome, this study offers valuable insights for the development of future investigations into Citrus aurantium as a complementary resource in the management of anxiety. A promising avenue for methodological improvement lies in the implementation of technologies that allow for standardization and greater control of essential oil inhalation, ensuring adherence to the established time and dosage.

A study published by Gabriel Chaves Neto et al. (2017) investigated the anxiolytic effect of *Citrus aurantium* essential oil in abstinent crack users, using controlled nebulization and measures such as IDATE-E, HAS, and physiological parameters (BP, HR, skin conductance). The results showed a significant reduction in anxiety in groups that inhaled the oil, as a control for levels of discomfort and cognitive impairment, demonstrating results consistent with psychophysiological markers. Comparing the studies, we found that the research evaluated here used a sample of healthy university students (with high emotional variability), with n=42, a sample similar to the comparative study, but less homogeneous (medication, psychotherapy, insomnia, etc.). Although there was no significant difference, the experimental group tended to have a higher average score.

This divergence between studies can be attributed to the method of administration (nebulization *versus* manual inhalation), the scale used (IDATE *versus* SAS), the population (patients with clinical anxiety *versus* healthy population), and finally the environment (controlled experimental *versus* complex environment). The use of nebulizers or ultrasonic diffusers represents an advantageous alternative. Such devices have been shown to be effective in the controlled administration of substances, as demonstrated in a study that evaluated **the** anxiolytic effect of *Citrus aurantium* L**.** in crack users through nebulization, indicating a promising route for dose and absorption control [21].

This instrumental precision favors more effective bioavailability of the active components in the central nervous system, culminating in more consistent and replicable therapeutic responses and mitigating problems of adherence to exposure time. For future scientific endeavors, we suggest comparing the efficacy of manual inhalation (as used in this study) with device-assisted inhalation, evaluating not only anxiety reduction but also the pharmacokinetics of the inhaled compounds and participant adherence. It is also recommended to explore different oil concentrations, durations, and frequencies of exposure, as well as to conduct studies with more homogeneous samples (free of concomitant treatments) or with more robust control designs to isolate the effects. The inclusion of stress biomarkers (such as salivary cortisol) in conjunction with psychometric scales could also enrich the objectivity of data on neurophysiological impacts.

Another highlight in the lifestyle analysis was the observation of often intense routines. Although the general diet was considered adequate by most, sleep patterns were consistently impaired, with common complaints of irregularity or poor sleep quality. The scientific literature corroborates the relevance of this observation, indicating that sleep deprivation, even if brief, can significantly increase anxiety levels. This phenomenon is attributed to the impairment of the functionality of brain regions involved in emotional balance, such as the prefrontal cortex, and to the intensification of activity in the amygdala, a brain area associated with stress and fear reactions. Additionally, evidence suggests that deep sleep plays a crucial role in the brain's processing of emotions and in maintaining emotional stability for the following day [22-23]. In conclusion, the results obtained in this study, considering both the entire sample and the placebo subgroup, suggest that the administration of *Citrus aurantium* essential oil alone was not effective in promoting a significant reduction in the participants' anxiety levels. Despite the lack of proven

effectiveness in reducing anxiety, this research makes a valuable contribution to the body of scientific evidence by pointing out practical limitations in the application of aromatherapy in an academic context and under uncontrolled exposure conditions.

Critical analysis and discussion of possible causes for the negative result, such as the manual form of inhalation, the post-test timing for data collection, and the lack of precise control over the inhaled dose, demonstrate the need for further studies in health or aromatherapy journals, with an emphasis on methodological lessons and implications for future clinical trials with essential oils.Future investigations using nebulizers or ultrasonic diffusers that allow standardization of the dose and exposure time to the essential oil. The application of scales that are more sensitive to acute changes, such as the IDADE-E, and at different times during the test, such as before and after the assessment, in order to capture real variations in the anxious state, and even the application of the sample and stratification of subgroups, considering the emotional profile, sleep pattern, and history of self-reported anxiety, which may interfere with the individual response to the intervention.

It is reasonable to infer that factors inherent to the methodology, such as the period of exposure to the oil and the method of application used, or even the particular characteristics of the group of participants, influenced the observed outcomes. It was also observed that the participants used various strategies to cope with anxiety, some of which proved beneficial in relieving symptoms, while others did not alter the symptomatic picture. This scenario emphasizes the complexity of anxiety and the importance of considering the interrelationship of multiple psychosocial, individual, and environmental factors in modulating its symptoms.

Thus, it is clear that the management of anxiety, in many contexts, requires a holistic and integrated approach that is not limited to the exclusive intervention of substances with anxiolytic properties.

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Conflicts of Interest: None.

Supplementary Materials: None.

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