

Efeito ansiolítico de óleos essenciais: uma revisão sobre estudos pré-clínicos e clínicos

Ansiolytic effect of essential oils: a review on pre-clinical and clinical studies

Efecto ansiolítico de los aceites esenciales: una revisión de estudios preclínicos y clínicos

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Resumo

A ansiedade participa da defesa natural do organismo, no entanto, ela pode ser patológica, quando os sintomas passam a interferir na rotina do indivíduo, causando perda da qualidade de vida. Os óleos essenciais (OEs) são metabólitos secundários com inúmeras atividades farmacológicas e biológicas, onde podemos destacar a atividade ansiolítica. Desta forma, objetivou-se realizar uma revisão sistemática dos últimos 9 anos na grande área de farmacologia e toxicologia, e verificar quais OEs apresentam atividade ansiolítica, quais os ensaios foram realizados e os possíveis mecanismos envolvidos. Realizou-se uma revisão bibliográfica com natureza quali-quantitativa, utilizando-se a base de dados Scopus, onde foram selecionados estudos originais, publicados entre janeiro de 2010 a 10 de agosto de 2019, do tipo ensaio pré-clínico e clínico que apresentam resultados referentes à atividade ansiolítica de EOs, sendo excluídos aqueles estudos que não compreendiam a área de farmacologia e/ou toxicologia. Ao final desta análise, dos 71 trabalhos encontrados, 18 foram selecionados para compor esta revisão. Esta revisão possibilitou visualizar o panorama dos estudos pré-clínicos e clínicos para avaliação da ansiedade com a utilização de OEs dos últimos 9 anos, apontando que além do OE de *Lavandula angustifolia*, outros OEs também podem ser promissores, como por exemplo os OEs obtidos de espécies do gênero *Citrus*. No entanto, verifica-se a escassez de estudos clínicos para a comprovação da eficácia e segurança do uso destes OEs no tratamento da ansiedade, o que possibilita a abertura de pesquisas nesta área para o desenvolvimento e lançamento de novos ansiolíticos naturais.

Palavras-chave: Ansiedade; Farmacologia; Aromaterapia.

Abstract

Anxiety participates in the body's natural defense, however, it can be pathological, when symptoms start to interfere in the individual's routine, causing loss of quality of life. Essential oils (EOs) are secondary metabolites with numerous pharmacological and biological activities, where we can highlight anxiolytic activity. Thus, the objective was to carry out a systematic review of the last 9 years in the large area of pharmacology and toxicology, and to verify which EOs have anxiolytic activity, which tests were performed and the possible

mechanisms involved. A bibliographic review with a qualitative and quantitative nature was carried out, using the Scopus database, where original studies were published, published between January 2010 and August 10, 2019, of the pre-clinical and clinical trial type that present results referring to anxiolytic activity of EOs, excluding those studies that did not understand the area of pharmacology and / or toxicology. At the end of this analysis, of the 71 papers found, 18 were selected to compose this review. This review made it possible to visualize the panorama of preclinical and clinical studies for assessing anxiety with the use of OEs in the last 9 years, pointing out that in addition to the EO of *Lavandula angustifolia*, other EOs can also be promising, such as the EOs obtained from species of the genus *Citrus*. However, there is a scarcity of clinical studies to prove the efficacy and safety of the use of these EOs in the treatment of anxiety, which allows the opening of research in this area for the development and launch of new natural anxiolytics.

Keywords: Anxiety; Pharmacology; Aromatherapy.

Resumen

La ansiedad participa en la defensa natural del organismo, sin embargo, puede ser patológica, cuando los síntomas comienzan a interferir en la rutina del individuo, provocando pérdida de calidad de vida. Los aceites esenciales (AEs) son metabolitos secundarios con numerosas actividades farmacológicas y biológicas, entre las que podemos destacar la actividad ansiolítica. Así, el objetivo era realizar una revisión sistemática de los últimos 9 años en el amplio ámbito de la farmacología y toxicología, y comprobar qué AE tienen actividad ansiolítica, qué pruebas se han realizado y los posibles mecanismos implicados. Se realizó una revisión bibliográfica de carácter cualitativo y cuantitativo, utilizando la base de datos Scopus, donde se publicaron estudios originales, publicados entre enero de 2010 y el 10 de agosto de 2019, del tipo de ensayo clínico y preclínico que presentan resultados referido a la actividad ansiolítica de los AEs, excluyendo aquellos estudios que no comprendieron el área de farmacología y / o toxicología. Al final de este análisis, de los 71 artículos encontrados, 18 fueron seleccionados para componer esta revisión. Esta revisión permitió visualizar el panorama de los estudios preclínicos y clínicos para evaluar la ansiedad con el uso de AEs en los últimos 9 años, señalando que además de la AE de *Lavandula angustifolia*, otras AEs también pueden ser prometedoras, como los AEs obtenidas de especies del género *Citrus*. Sin embargo, existe una escasez de estudios clínicos que demuestren la eficacia y seguridad del uso de estos AEs en el tratamiento de la ansiedad, lo que permite la apertura de la investigación en esta área para el desarrollo y lanzamiento de nuevos ansiolíticos naturales.

Palabras clave: Ansiedad; Farmacología; Aromaterapia.

1. Introduction

Generalized anxiety disorder (GAD) is characterized by intense anxiety with physical and somatic symptoms (Sousa et al., 2015). Anxiety is defined by tension or discomfort accompanied by a feeling of emptiness, fear and apprehension due to the anticipation of risks that the individual may or may not take. Despite being a type of natural defense of the organism, it can be pathological when it starts to interfere in the daily lives of individuals, in a way to disrupt their quality of life and their daily performance, jeopardizing the personal and professional relationships (Allen; Leonard; Swedo, 1995; Sousa et al., 2015). Therefore, the best way to assess whether this anxiety is normal or pathological is to check the duration of this state (Castillo et al., 2000; Ribeiro et al., 2020).

The world population has a prevalence of generalized anxiety disorder (GAD) of 3.6%, with this number increasing dramatically reaching 5.6% in the American continent and 9.3% in Brazil (WHO, 2017). As in other pathologies that affect the central nervous system, the GAD is more common in women (4.6%) than in men (2.6%) (Who, 2017).

In 2016, for example, there was a significant increase in records of the number of sick leave and grants for disease aid in Teresina, Piauí, Brazil (Fernandes, et al., 2018). In addition, according to Ribeiro et al. (2019) there is a high prevalence of GAD as a cause of sick leave and costs with disease. Furthermore, the authors draw attention to the negative impacts on the lives of these workers, especially when psychological manifestations interfere in family and social life, as well as in daily life, reducing their potential at work (Sousa et al., 2015; Ribeiro et al., 2019). Thus, it is extremely important to have an effective and safe treatment.

Currently, the pharmacological treatment of GAD is done with the use of benzodiazepines and antidepressants (Sousa et al., 2015). Benzodiazepines, which are also classified as anxiolytics, are psychotropic drugs used to treat anxiety symptoms, modulating emotions, mood and behavior (Ferrazza et al., 2010). However, once they act on the central nervous system (Griffin et al., 2013), these drugs can cause dependence, especially if the use occurs irrationally; causing harm to the patient's health such as amnesia, sedation, cognitive changes and sexual dysfunctions (Sousa et al., 2015), which has led to a search for other treatment options that are less harmful.

According to the World Health Organization (WHO), alternative medicine is one of the main practices of primary health care in developing countries, due to limited access to conventional health services, especially in rural regions. In addition, in many countries, the use of alternative practices still prevails for cultural reasons or as a complement to health (Who, 2013).

Aromatherapy is a procedure listed on the list of Integrative and Complementary Practices in Health (PICS) and offered by the Brazilian Unified Health System (SUS) to the population. This therapy uses essential oils from plants with healing properties, in order to promote health, physical, and mental well-being (Brazil, 2019). Essential oils (EO) are secondary metabolites extracted from the most diverse plant organs (leaves, flowers, pericarps, roots) and have aroused interest in the area of aromatherapy for providing emotional changes, such as tranquility, euphoria and vigor, in humans and animals.

Therefore, EOs can be used concomitantly with conventional treatments as a complement, as they can reduce the undesirable symptoms of drugs. In addition, studies show that EOs have several therapeutic benefits, such as in the treatment of Alzheimer's disease and as anxiolytics, acting on the central nervous system (Shu, 1998; Faustino; Almeida; Andreatini, 2010). An example is the EO of *Lavandula angustifolia* Mill. (Lamiaceae) which has been patented as an anxiolytic Silexan®, after several phytochemical studies and preclinical and clinical trials (Marcas, 2008).

Due to the problems caused by pathological anxiety and the importance of new natural drugs with less undesirable effects, the objective was to carry out a survey of the studies of the last nine years in the great area of pharmacology and toxicology and to verify which OEs have anxiolytic activity, as well as tests performed and the possible mechanisms of action involved.

2. Methodology

For this research, a systematic and qualitative and quantitative bibliographic review was carried out (Pereira A.S. et al. 2018). Therefore, the inclusion and exclusion criteria adopted are described below, as well as the search strategy.

Criteria for inclusion and exclusion of studies

Original studies were used, such as pre-clinical and clinical trials that show results regarding the anxiolytic activity of essential oils. The other inclusion criteria were: open access documents, type of article, with publication date between January 2010 and August 10, 2019. This time frame is justified by the fact that the search took place on August 10, 2019. Studies that did not belong to the area of pharmacology and / or toxicology were also excluded.

Search strategy

The search for articles was carried out in the Scopus database, using the keywords “Essential oil” combined with “Anxiolytic activity”. A first evaluation was carried out, based on the titles and summary of articles, and those that did not meet the inclusion criteria or presented any of the exclusion criteria were rejected. Secondly, the full text was analyzed, when the study cannot be included or rejected with certainty. In addition, previous review articles were included, as well as other articles that addressed the topic, to complement this review.

3. Results and discussion

At the end of this analysis, of the 71 considered studies, 18 were selected to compose this review, as they were in accordance with the inclusion and exclusion criteria.

Of these 18 studies, only three are clinical studies, highlighting the scarcity of these studies in the large area of pharmacology and / toxicology. In addition, only the OE of *Lavandula angustifolia* Mill. (Lamiaceae) was used in these studies. Next, we will better discuss the data found.

Overview of work

These studies report anxiolytic activity for EOs of 13 plant species, which are: *Liquidambar orientalis* Mill. (Altingiaceae); *Annona vepretorum* Mart. (Annonaceae); *Ferula assa-foetida* L. (Apiaceae); *Lavandula angustifolia*; *Piper guineense* Schumach. & Thonn. (Piperaceae); *Cymbopogon citratus* (DC.) Stapf. (Poaceae); *Citrus x aurantium* L. (Rutaceae),

C. bergamia Risso (Rutaceae), *C. limon* (L.) Burm.f. (Rutaceae), *C. sinensis* (L.) Osbeck (Rutaceae), *Spiranthera odoratissima* A.St.-Hil. (Rutaceae); *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilsson (Verbenaceae) chemotype II and *Curcuma longa* L. (Zingiberaceae).

Most studies (33.33%) reported anxiolytic activity for the EO of *Lavandula angustifolia*. Of these studies, two of them carried out clinical studies with Silexan® (Baldinger et al., 2014; Kasper et al., 2014). The most widely used route of administration of EOs in the studies was by inhalation (44%), followed by oral (33%) and intraperitoneal (22%). Among the most used anxiety models we have the elevated plus maze test (38%), followed by the open field (27%) and light and dark (22%). It was noted that most studies used more than one anxiety model to verify the anxiolytic activity.

Essential Oils with anxiolytic activity - Pre-clinical studies

Table 1 shows the pre-clinical studies that investigated the anxiolytic activity of EOs.

Table 1. Essential oils with anxiolytic activity: pre-clinical trials.

References	Essencial Oil	Family	Animal Model	Administration	Anxiety Model	Observation
Umezu, (2000)	<i>Lavandula angustifolia</i>	Lamiaceae	Ma. ICR mice	Ip.	Conflict – Geller	Anxiolytic effect
Leite et al. (2008)	<i>Citrus x aurantium</i>	Rutaceae	Rats	In.	Open Field Test (OFT)	Anxiolytic effect
Costa et al. (2011)	<i>Cymbopogon citratus</i>	Poaceae	Adult Swiss ma. Mice	Oral	Elevated plus maze test (EPM) Light-dark test (LDT)	Anxiolytic effect
Alqasoumi, (2012)	<i>Ferula assa-foetida</i>	Apiaceae	Healthy male adult Swiss albino mice and Wistar albino rats	Oral	Marble Burying test (MBT)	No effect
Galdino et al. (2012)	<i>Spiranthera odoratissima</i>	Rutaceae	Ma. adult Swiss mice	Oral	Elevated plus maze test (EPM) Hole-board test	Anxiolytic effect
Hatano et al. (2012)	<i>Lippia alba</i> quimiotipo II	Verbenaceae	Ma. Wistar rats	Ip.	Light-dark test (LDT)	Anxiolytic effect
Chioca et al. (2013)	<i>Lavandula angustifolia</i>	Lamiaceae	Adult male Swiss albino mice	In.	Elevated T maze (ETM)	Anxiolytic effect
Tankam & Ito (2013)	<i>Piper guineense</i>	Piperaceae	Four-week-old ma. ddY mice	In.	Marble Burying test (MBT)	Anxiolytic effect
Viana et al. (2016)	<i>Citrus limon</i>	Rutaceae	Ma. Albino Swiss mice	In.	Elevated plus maze test (EPM)	Anxiolytic effect
Oyemitan et al. (2017)	<i>Curcuma longa</i>	Zingiberaceae	Swiss-Webster mice of both sexes	Ip.	Open Field Test (OFT)	Anxiolytic effect
Caputo et al. (2018)	<i>Lavandula angustifolia</i>	Lamiaceae	Ma. mice of the OF1 strain	Ip.	Hole-board	Anxiolytic effect
Liang et al. (2018)	<i>Liquidambar orientalis</i>	Altingiaceae	Ma. ICR mice	In.	Elevated plus maze test (EPM)	Anxiolytic effect
Wolffenbüttel et al. (2018)	<i>Citrus x aurantium</i> <i>Citrus sinensis</i>	Rutaceae	Ma. adult albino mice	In.	Open Field Test (OFT)	Anxiolytic effect
Diniz et al. (2019)	<i>Annona vepretorum</i>	Annonaceae	Adult (3 months old) ma. albino Swiss mice	Oral	Light-dark test (LDT)	Anxiolytic effect
					Open Field Test (OFT)	Anxiolytic effect
					Elevated plus maze test (EPM)	Anxiolytic effect
					Hole-board	Anxiolytic effect

Legend: Ma: Male; Ip: Intraperitoneal; In: Inalation.

Source: Authors (2020).

Note in Table 1 that the EO of *Lavandula angustifolia* was the most used in these preclinical studies, however, it is noticed that other EO also had this effect. Another important fact to note is the route of administration, where the most used was inhalation. In addition, there are different tests and animal models to check anxiolytic activity.

The EOs of *Lavandula angustifolia*, *Citrus x aurantium*, *Lippia alba* chemotype II and *Piper guineense* showed anxiolytic effect greater than or equal to the allopathic Diazepam, in which the EOs were administered from different routes: the first three being administered intraperitoneally and the last one through inhalation (Umezu, 2000; Leite et al., 2008; Hatano et al., 2012; Tankam & Ito, 2013).

During the evaluation of the anxiolytic effects of the EO of *Lavandula angustifolia*, Caputo et al. (2018) found that an acute intraperitoneal injection of this essential oil (200 mg/kg) reduced motor activity in rats, without any anxiolytic effect, although it has shown an increase in social interaction. In addition, the acute administration of this EO in stressed rats after a social defeat encounter, was effective in reducing the levels of anxiety and reversing the social aversion.

The EO of *Piper guineense* at 4.0×10^{-3} mg concentration demonstrated a better sedative action when compared to the EO of *Lavandula angustifolia* at 4.0×10^{-4} mg (Tankam & Ito, 2013). However, it was observed that the anxiolytic effect of OE of *P. guineense* was better than the control (Diazepam at 0.5 mg) increasing the residence time in the light area during the light/dark test at the concentration of $4, 0 \times 10^{-6}$ mg per box. In addition, comparing the areas under the curve (AUC) that represent the total locomotor activity (TLA), calculated from the pattern with the observed TLA, the *P. guineense* OE showed anxiolytic activity considered as moderate by the authors (Tankam & Ito, 2013). In this sense, from the different results pointed out in these studies, it may be necessary to adapt some factors, such as form of administration and exposure time, once these can directly influence on the anxiolytic effects.

The EO of *Cymbopogon citratus* did not show anxiolytic activity in the marble burying test (MBT), however, there was anxiolytic activity in the light and dark box test (LDB). This result may indicate that the form of administration and the type of anxiety model (test) may influence in the results of these tests. According to Umezu (2000), the essential oil of *Boswellia thurifera* Roxb Ex Fleming. (Burceraceae) decreased the response time during the safety period of the Geller-type conflict test but did not increase the response rate even at high doses. Therefore, the authors preferred not to state that this EO had an anxiolytic effect,

while the essential oil of *Lavandula angustifolia* was reported by have an anxiolytic effect, as it increased the response rate in the same way as the anxiolytic Diazepam (Umezu, 2000).

The EO of *Citrus x aurantium* at a concentration of 2.5% showed an anxiolytic effect, which increased the permanence time of the mice in the EPM test and the interaction time during the OFT, in which the interaction time was better than that of the control with Diazepam (1.5 mg/kg) (Leite et al., 2008). The EO of *Cymbopogon citratus* at a dose of 2.5 mg/kg, showed anxiolytic activity and at a dose of 10 mg/kg in the LDB test it showed a synergistic effect when administered with ineffective doses of Diazepam (0.25 mg/kg) (Costa et al 2011). The result found by Costa et al. (2011), points out to a possible alternative for the "weaning" of patients who have been using Diazepam for a long time, since the anxiolytic activity persisted, influenced by the synergism between the EO with the allopathic.

The EO of *Spiranthera odoratissima* showed anxiolytic activity for EPM, LDB and hole-board tests. In addition, in none of these tests the motor activity was compromised (Galdino et al., 2012). Viana et al. (2016) verified anxiolytic activity for all doses (100, 200, and 400 µL) of *Citrus limon* EO compared to the Diazepam control (1.5 mg/kg) for the OFT, without altering the animals' motor performance. Furthermore, this EO showed no toxicity in mice. The EPM test corroborated with the data from the previous test in two doses tested (200 and 400µL) of this EO (Viana et al., 2016). Han et al. (2017) investigated the safety of *Citrus bergamia* EO inhalation in a pilot clinical study. The authors found that after 15 minutes of exposure, the participants' positive feelings increased 17% compared to the control group, providing preliminary evidence of efficacy and safety of inhaling this EO in mental well-being at a mental health treatment center.

Oyemitan et al. (2017) while assessing the anxiolytic activity of *Curcuma longa*, found that this EO was slightly toxic when administered orally and moderately toxic by intraperitoneal route in mice. Despite this fact, anxiolytic and sedative activity and anticonvulsant effects were verified. These authors also found that this EO has significant anxiolytic activity with dose-dependent concentrations of 50-100 mg/kg. In this experiment, the rats behaved similarly to the group treated with Diazepam (1 mg/kg), however the dose of 200 mg/kg led to a reduction in head diving in the hole-board test. It was also possible to verify the anxiolytic activity of this oil in the EPM test.

Liang et al. (2018) demonstrated that repeated inhalation of the EO of *Liquidambar orientalis* by mice exposed to acute stress, exerted significant antidepressant and anxiolytic activities. In the same year Wolffenbüttel et al. (2018) while assessing the anxiolytic activity of the EO of *Citrus x arantium* and *Citrus sinensis*, reported that only the inhalation of C.

sinensis essential oil at the concentration of 10% had the sedative and anxiolytic effects. In the following year, Diniz et al. (2019) found that *Annona vepretorum* EO had anxiolytic, sedative, antiepileptic and antidepressant effects.

The EO obtained from *Ferula assa-foetida* showed a dose-dependent effect for anxiolytic activity, in addition to a sedative effect at high doses (Alqasoumi, 2012). This effect may indicate that it may contain a greater amount of the compounds responsible for the anxiolytic effects when administered in a higher dose. Significant anxiolytic activity was evidenced at a dose of 250 mg/kg for the EPM test and according to Alqasoumi (2012) these results suggest that this EO may be an alternative for the treatment of anxiety disorders. However, further studies are needed to assess its accuracy and prove the safety and effectiveness of this EO.

According to the results of this review, only the EO of lavender has a patented product, however, other EOs are also potentially promising, such as the *Citrus x aurantium*, *Lippia alba* chemotype type II and *Piper guineense* essential oils. In these studies, the authors reported their potential use, concomitant with allopathic drugs or even to replace the one used worldwide (Diazepam), thus avoiding a series of side effects (Leite et al., 2008; Hatara et al., 2012; Chioca et al., 2013). Therefore, it is believed that new toxicity and safety studies, as well as clinical studies, may contribute to the release of new patent applications and the new formulation of products with these EOs.

Reviewing these articles, it was found that the anxiolytic activity of these EOs varied according to different factors such as dose, anxiety model, animal model and route of administration. Consequently, the determination of anxiolytic activity may be influenced if these parameters are not well defined.

Essential Oils with anxiolytic activity – clinical studies

Table 2 shows the clinical studies that investigated the anxiolytic activity of Eos.

Table 2. Essential oils with anxiolytic activity: clinical trials.

References	Essencial Oil	Family	Animal Model	Administration	Anxiety Model	Observation
Kasper et al. (2014)	<i>Lavandula angustifolia</i>	Lamiaceae	Adult men (GAD)	Oral	randomized, double-blind, double-dummy, multicentre trial with four parallel groups	Anxiolytic effect
Baldinger et al. (2014)	<i>Lavandula angustifolia</i>	Lamiaceae	Healthy adult men	Oral	double-blind, randomized, placebo-controlled, cross-over trial	Anxiolytic effect
Han et al. (2017)	<i>Citrus bergamia</i>	Rutaceae	Men and women	In.	Positive and Negative Affect Schedule (PANAS)	Anxiolytic effect

Legend: GAD: Generalized anxiety disorder; In: Inhalation.
 Source: Authors (2019).

From Table 2, there are few clinical studies that investigated the anxiolytic activity of OE in this period. In addition, all studies investigated the anxiolytic activity of the OE of *Lavandula angustifolia*, where in two studies this OE was administered orally.

There are only three clinical studies that have evaluated the anxiolytic activity of EOs. In the first one, men diagnosed with generalized anxiety disorder (GAD) ingested 80 or 160 mg capsules of Silexan® daily for a period of 10 weeks (Kasper et al., 2014) and it demonstrated a moderate anxiolytic effect. This effect may have been directly influenced by the clinical situation of patients with GAD, as well as by the time of exposure (10 weeks). In addition, Kasper et al. (2014) demonstrated that Silexan® had anxiolytic activity and had a pronounced antidepressant effect and improved overall mental health and quality related to the health of life. In addition, it showed better activity when compared to the placebo group and inferior activity when compared to control with 20 mg of paroxetine (Kasper et al., 2014).

In another clinical study, in which Silexan® was also used, Baldinger et al. (2014) found that the daily administration of 160mg of Silexan® caused a reduction in anxiety levels in healthy adult men after 8 weeks. These results indicate that men diagnosed with GAD possibly need more time and/or higher doses at first, so that there are significant improvements in their clinical picture. Despite this, the anxiolytic activity was considered as moderate by the authors. It is an excellent result and demonstrates the feasibility of using this herbal medicine in substitution to the conventional allopathic drugs linked to medical monitoring and other therapies.

Chemical composition of essential essentials

The essential oils have a high complexity of molecules in their composition and these compounds are responsible for therapeutic activity, alone or by synergism between the molecules. In this sense, it is important to verify which compounds are present in the EOs that showed anxiolytic activity. The chemical composition of the EOs is shown in Table 3.

Table 3. Chemical composition of essential oils with anxiolytic activity.

References	Essencial Oil	Chemical composition > 20%
Umezu, (2000)	<i>Lavandula angustifolia</i>	not performed
Leite et al. (2008)	<i>Citrus x aurantium</i>	limonene (96,24%) and myrcene (2,24%),
Costa et al. (2011)	<i>Cymbopogon citratus</i>	citral (71,3 %)
Alqasoumi, (2012)	<i>Ferula assa-foetida</i>	not performed
Galdino et al. (2012)	<i>Spiranthera odoratissima</i>	β - caryophyllene (20,6 %)
Hatano et al. (2012)	<i>Lippia alba</i> quimiotipo II	carvone (54,6 %) and limonene (23,1 %)
Chioca et al. (2013)	<i>Lavandula angustifolia</i>	linalyl acetate (53, 5%) and linalool (46,5 %)
Tankam & Ito (2013)	<i>Piper guineense</i>	linalool (41,8%)
Kasper et al. (2014)	<i>Lavandula angustifolia</i> Silexan ®	not performed
Viana et al. (2016)	<i>Citrus limon</i>	not performed
Han et al. (2017)	<i>Citrus bergamia</i>	limonene (36,0 %) e linalyl acetate (31,0 %)
Oyemitan et al. (2017)	<i>Curcuma longa</i>	turmerone (35,9 %)
Caputo et al. (2018)	<i>Lavandula angustifolia</i>	not performed
Liang et al. (2018)	<i>Liquidambar orientalis</i>	not performed
Wolffenbüttel et al. (2018)	<i>Citrus x aurantium</i>	linalool (22,4 %) and linalyl acetate (28,0 %)
	<i>Citrus sinensis</i>	limonene (78,3 %)
Diniz et al. (2019)	<i>Annona vepretorum</i>	(E)- β -ocimene (42,6 %)

Source: Authors (2020).

Analyzing the Table 3, it appears that among the compounds found in the EOs, the most commonly found were limonene, linalool and linalyl acetate. Therefore, it is believed that these are the main compounds involved in anxiolytic activity. In this sense, it is important to discuss some information about limonene and linalool, in order to verify chemical similarities and mechanism of action.

Linalool

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is an acyclic monoterpenoid (C₁₀H₁₈O) found in several essential oils such as *Lavandula angustifolia*, *Citrus x aurantium*, *Lippia alba*, among other plant species (Hatano et al. 2012; Caputo et al. 2018; Wolffenbüttel et al. 2018).

Its chemical structure has a chiral carbon and, for this reason, there may be two isomers: (R) - (-) - linalool (licareol) and (S) - (+) - linalool (coriandrol), which give it different olfactory perceptions. The concentrations of linalool, as well as its isomers, can vary widely in plants according to abiotic and biotic factors.

Over the years, several studies have reported the sedative effects related to linalool (Aprosoaie et al., 2014; Pereira, I. et al., 2018). Dos Santos et al. (2018), when verifying the antidepressant properties of linalool administered intraperitoneally in mice, by the OFT, EPM and forced swimming tests. The results showed that EOs rich in linalool and the isolated linalool itself did not cause a depressive effect in the Central Nervous System (CNS), since they did not interfere with the spontaneous locomotion or short-term memory of the animals in the inhibitory avoidance test (Dos Santos et al., 2018).

In a study performed by Cheng, Sheen and Chang (2015), the results of measurements of monoamines in the brain of mice revealed reductions in serotonin, dopamine and norepinephrine. These results were considered consistent with the anxiolytic effects in animal models. In addition, they found that the R and S isomers of linalool also have anxiolytic properties without side effects, as they did not affect locomotor activity, drowsiness or unusual involuntary movements. Thus, the authors support its potential use in the treatment of anxiety disorders (Cheng; Sheen and Chang, 2015).

Harada et al. (2018), also observed the anxiolytic effect of linalool and reported that this compound did not compromise locomotor activity in mice. In the same year, Caputo et al. (2018) found that acute or chronic administration of linalool before an episode of social defeat was able to reverse social aversion, acting as an antidepressant agent.

Takahashi et al. (2011) described the anxiolytic effect of EOs obtained from six different species of *Lavandula* L., tested by inhalation using the EPM test in mice. The results indicated that the linalool effect was potentiated by linalyl acetate, where these compounds acted in synergism.

Another substance is the linalool oxide, which can be found in some EOs and can also be formed from a natural oxidation process. From tests of EPM and OFT anxiety models, this

compound showed an anxiolytic effect by intraperitoneal application in mice. In addition, linalool oxide did not interfere with motor activity, as well as linalool in other studies (Souto-Maior et al., 2011).

Therefore, it appears that linalool and its isomers licareol and coriandrol are responsible for the anxiolytic activity of EOs, in which these compounds are present. In this sense, new studies should be carried out in order to support the formulation of anxiolytic drugs that contain these compounds.

Limonene

Limonene (4-isoprenyl-1-methyl-cyclohexene) is a monocyclic monoterpene that is part of the structure of more than 300 vegetables (Burdock, 1995). This compound also has a chiral carbon and can be found in the form of stereoisomers (R)-(+)-limonene and (S)-(-)-limonene.

(R)-(+)-limonene is the most commonly found isomer in nature, being common in EOs of oranges, bergamots, tangerines and lemon species, while (S)-(-)-limonene is reported in species of the genus *Cymbopogon*, such as in the EO of *Cymbopogon citratus* (Mosandl et al., 1995).

Several articles have stated the anxiolytic effects of (R)-(+)-limonene. In a test performed in a EPM test with mice, this compound was shown to be effective at concentrations of 0.5% and 1.0%, while administered by inhalation (Lima et al., 2013). This compound also had an anxiolytic effect similar to that observed with Diazepam (1 mg/kg, i.p.) (Deng et al., 2010).

In another study, limonene was detected as the main component found in the plasma of mice after inhaling the EO of *Citrus sinensis*. The authors related the anxiolytic effect of this essential oil due to the presence of limonene (Wolffenbüttel et al., 2018).

Therefore, it appears that limonene and its stereoisomers (R)-(+)-limonene and (S)-(-)-limonene are responsible for the anxiolytic activity of EOs, in which they are present.

Mechanisms of action of essential oils in anxiolytic activity

Some studies have tried to demonstrate the mechanism of action of anxiolytic activity induced by different EOs. Galdino et al. (2012) verified that the anxiolytic effect mediated by EO of *Spiranthera odoratissima* was blocked by pretreatment with NAN-190, a 5-HT_{1A}

receptor antagonist, but was not blocked by pretreatment with flumazenil, a GABA_A receptor antagonist. These results suggest that the anxiolytic effect is dependent on the activation of 5-HT_{1A} receptors. Diniz et al. (2019) evaluated the anxiolytic effect of the EO of *Annona vepretorum* in mice and demonstrated that the pretreatment with flumazenil inhibited the action of the EO, suggesting that the effect of this EO is due to activation of GABA_A receptors.

Chioca et al., (2013) in experiments carried out with mice demonstrated that the pretreatment with WAY 100635, a 5-HT_{1A} receptor antagonist, blocked the anxiolytic effect of the EO of *Lavandula angustifolia*, inferring that the effect is dependent on the activation of these receptors. The only clinical study was carried out by Baldinger et al. (2014) who demonstrated by positron emission tomography that after 8 weeks of treatment with Silexan® containing 160mg of *Lavandula angustifolia* there was interaction of this substance with 5-HT_{1A} receptors

From these studies, it can be inferred that, depending on the EO, the anxiolytic effect can occur by different mechanisms, such as by activating the gabaergic system or by stimulating the serotonergic system. This diversity of mechanisms can be explained by the fact that EOs have a high chemical complexity and this characteristic can make them more efficient and with less adverse effects than conventional drugs.

This review made it possible to visualize the panorama of preclinical and clinical studies for assessing anxiety with the use of EOs in the last 9 years. Thus, it was noted that, among the species, the EO of *Lavandula angustifolia* stands out for the largest number of studies and the existence of a commercial product for the treatment of anxiety. It is also noticed that there are other promising EOs for controlling anxiety, such as those obtained from *Citrus* species. However, further studies are needed to prove the efficacy and safety of using these EOs in the treatment of anxiety.

4. Conclusion

The present review highlights the scarcity of clinical studies for the treatment of anxiety in the past nine years in the area of pharmacology and toxicology. However, this study contributes by pointing out the existence of other essential oils in addition to *Lavandula angustifolia*, such as *Citrus* oils, which also have shown to be potentially viable for the treatment of anxiety.

Therefore, this work encourages the development of new research in this area, mainly of clinical studies for the development and launch of new products based on essential oils for the treatment of anxiety.

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