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Revisión | Review

Pharmacological effects of different chemotypes of *Lippia alba* (Mill.) N.E. Brown

[Efectos farmacológicos de diferentes quimiotipos de *Lippia alba* (Mill.) N.E. Brown]

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Abstract: The *Lippia alba* species consists of an aromatic plant used in Brazilian traditional medical practice and in the medical practice of several countries as well. Presenting a wide variability in its essential oil chemical composition, the *Lippia alba* is classified in chemotypes, or chemical races, according to the major constituents contained in its essential oil. Considering the qualitative and quantitative distribution of the components in the essential oil affect directly its pharmacological properties, which are presented in the medicinal species, this paper proposes a scientific literature review to correlate both biological and pharmacological properties presented by *L. alba* according to its chemical constitution.

Keywords: *Lippia alba*; Chemotypes; Erva-cidreira; Essential oil.

Resumen: *Lippia alba* es una planta aromática utilizada en la medicina tradicional de Brasil y de varios países. Con una gran variabilidad en la composición química de su aceite esencial, se clasifica en quimiotipos, o razas químicas, de acuerdo con los constituyentes mayoritarios presentes en el aceite esencial. Dado que la distribución cualitativa y cuantitativa de los componentes del aceite esencial afecta directamente a las propiedades farmacológicas presentadas por la especie medicinal, este trabajo propone realizar una revisión en la literatura científica para correlacionar las propiedades biológicas y farmacológicas de los quimiotipos presentes en el aceite esencial de la *L. alba*.

Palabras clave: *Lippia alba*; Quimiotipos; Erva-cidreira; Aceite esencial.

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INTRODUCTION

In Brazil, there are several species of plants validated as medicinal, among them, the “erva-cidreira” or “falsa-melissa”, also known as *Lippia alba* (mill.) N.E. Brown, appears in all regions throughout the country. The *Lippia* genus consists of 200 species widely distributed throughout South America, Central America and across Africa (Zoghbi *et al.*, 1998).

The *L. alba* is an aromatic plant known for its wide variability in its essential oil chemical composition (Tavares *et al.*, 2005). In general, the chemical composition of essential oils changes – both qualitatively and quantitatively – due soil conditions, rainfall, geographic region, variety, plant age and extraction method (Jerkovic *et al.*, 2001; Bagamboula *et al.*, 2004). However, some authors suggest that the chemotype, or the chemical race, determines the chemical composition of essential oils (Castro *et al.*, 2002; Simões *et al.*, 2003; Tavares *et al.*, 2005; Manica-Cattani *et al.*, 2009). The classification of aromatic plants in chemotypes is very important, since it affects directly the active components, and thus, the pharmacological properties presented in their derivatives, either extracts or essential oil.

Several studies were conducted with *L. alba* from different regions demonstrated a wide variability in morphology and its chemical constitution, and these variations could not be explained by soil and climate conditions. According to Simões (2003), different plants grown in identical soil had different products, whereas similar plants grown in completely different soil displayed similar constituents. Some studies highlight that the *L. alba* genetic variation is not related to its geographical area, but to its chemotype (Manica-Cattani *et al.*, 2009).

L. alba morphological and chemical variations allow distinction in several chemotypes, those according to the predominance of some constituents present in the essential oil. Several authors intended to classify the chemotypes, but there is no broad consensus about the topic. Matos (2007), for instance, classified *L. alba* in three chemotypes: I, II and III. The type I has citral-myrcene predominance; type II has citral-limonene predominance and type III has carvone-limonene predominance. Januzzi *et al.* (2011) identified five essential oil chemotypes of *L. alba* in the Central-West region, in the city of Brasilia, and

these are: limonene-citral, myrcene-citral, myrcenol, citral and linalool. Hennebelle *et al.* (2008), researching the essential oil of *L. alba* retrieved from different regions in France, suggested a classification for these oils in seven chemotypes, classifying the major components of chemotype I as citral, or linalool, or β -caryophyllene; chemotype II as tagetenone; the limonene chemotype III followed by the various amounts of carvone or monoterpenes ketones; the myrcene chemotype IV; the γ -terpinene chemotype V; the camphor-1 chemotype VI, cineole-8 and chemotype VII as estragole.

Regarding the wide variety of chemotypes identified to the *L. alba* and considering that it is a medicinal plant largely employed by the Brazilian population and by many other countries around the world, this paper proposes a scientific literature review to correlate both biological and pharmacological properties presented by *L. alba* according to its chemical constitution.

This systematic review was carried out in October 2017 and was performed through PubMed Medline, Scopus (Elsevier), Science Direct, SciELO and Lilacs via Virtual Health Library (Bireme) using several combinations of the following keywords *Lippia alba* and chemotypes. The manuscript selection was based on the inclusion criteria: articles published in English, Portuguese and Spanish and articles with keywords in the title, abstract or full-text. 127 articles were identified. However, 24 papers were indexed in two or more databases and were considered only once. Out of this total, 35 articles were selected as the others did not meet the inclusion criteria. For the selection of the manuscripts, three investigators first selected the articles according to title, then to abstract and then through an analysis of the full-text publication. Any disagreement was resolved through a consensus between the investigators. The resulting articles were manually reviewed with the goal of identifying and excluding the studies that did not fit the criteria described above.

THE PHARMACOLOGICAL ACTIONS OF *Lippia alba*

Activities in the Central Nervous System (CNS)

Experimental studies indicate that the essential oil of *L. alba* with high levels of carvone (around 55%) presents anxiolytic properties, supporting previous findings reporting the efficacy on repeated treatments

with carvone as a powerful depressant in the Central Nervous System. This specific study suggests that carvone possibly interacts with GABA receptors in the brain, inhibits neurons and modulate the behavior inhibition (Vale, 1999). There is evidence that carvone reaches certain brain areas, such as the cerebellar amygdala, the main cerebral area responsible for the integration of emotional responses and related to the recognition of potentially pleasant stimuli to the individual (Hatano *et al.*, 2012).

The citral-limonene chemotype essential oil, when injected intraperitoneally in rats, presented anxiolytic, sedative, antipyretic and myorelaxant effects in behavioral evaluation tests (Vale, 1999). In another study, the evaluation of isolated substances as citral, myrcene, and limonene, which are present in the essential oil of *L. alba*, exposed sedative and myorelaxant effects, in addition to the pentobarbital potentiation on rodents. However, these isolated substances do not present anxiolytic actions, suggesting that the activity must be a result of non-major components in the essential oil of the plant (Vale *et al.*, 2002).

Hennebelle *et al.* (2008) investigated the sedative properties of metabolites in the citral chemotype essential oil (EO) of *L. alba* and confirmed a weak to moderate action on the benzodiazepine receptors.

The anticonvulsant effects of essential oil in three chemotypes of *L. alba* (citral, myrcene, and limonene) were evaluated in a model of pentylenetetrazole-induced seizure in mice. The results demonstrated the three chemotypes evaluated increased the latency of pentylenetetrazole-induced seizures. It was also observed a significant increase of the anticonvulsant effect in the essential oils evaluated with diazepam. The survival rate increased from two to three times with essential oil and diazepam together in relation to the essential oil in the absence of diazepam. It was also demonstrated that citral creates an anticonvulsant effect in a smaller dose when compared to beta myrcene and limonene. Other authors suggest that these constituents present pharmacological effects similar to the diazepam (Viana *et al.*, 2000).

Mucolytic Action

Matos (2007), studying the essential oil from *L. alba* in the state of Ceará, observed that the plants with high levels of limonene-carvone (chemotype III)

present mucolytic action.

Analgesic and Anti-inflammatory Activities

Viana *et al.* (1998) analyzed the effects of essential oils from *L. alba* citral-limonene and carvone-limonene chemotypes, in models of a hot plate, acid-induced abdominal writhing and formalin test in mice; carrageenan-induced paw edema and dextran in rats. The results revealed that the two chemotypes tested caused inhibition to the acetic acid-induced writhing and showed significant results in the formalin test (inflammatory phase), indicating the possible participation of inflammatory mediators in the nociceptive effects from the evaluated oils. In the hot plate test, the latency for the thermal stimulus was increased only with the citral-limonene chemotype, this effect is reversed by the opioid agonist naloxone, thus suggesting a central analgesic action together with the opioid system. The carrageenan-induced paw edema or dextran test revealed a significant antiedematogenic effect for both chemotypes tested (Viana *et al.*, 1998).

Anesthetic action in fish

Studies of anesthesia induction in fish utilizing the Tambaqui species (*Colossoma macropomum*) proved that the citral chemotype essential oil from *L. alba*, whose major components were geranial (25,4%), neral (16,6%) and caryophyllene oxide (16%), induced fast anesthetic effect (<4 min) in 200 and 300 mg L⁻¹ doses (Batista *et al.*, 2018).

Activities in Gastrointestinal Tract: Gastroprotective activity

Some studies have proved the gastroprotector action of major constituents in the essential oil of *L. alba* species, among them the 1,8 cineole (Santos & Rao, 2001), citral (Ortiz *et al.*, 2010), limonene (Moraes *et al.*, 2009), linalool (Barocelli *et al.*, 2004).

A study carried with *L. alba* essential oil retrieved in the state of Ceará, myrcene-citral chemotype, showed mild antispasmodic property related to the citral component (Matos, 2007).

Antispasmodic

The antispasmodic effect was evaluated in the model of ileum and duodenum isolated organs in rats, from two *L. alba* chemotypes (citral and linalool), both grown in the region of La Plata, Argentina. The citral chemotype is constituted by geranial (19,5%),

carvone (16,7%) and neral (11,5%). The linalool chemotype presents linalool (64,2%) and 1-8 cineole (6%) in its composition. The results illustrate that the citral chemotype presented a potential five times greater than the linalool chemotype to inhibit muscarinic contractile responses. Both chemotypes interfered in calcium concentration (Ca^{2+} -influx), presenting an IC_{50} nearly 28 times higher than verapamil concentration. Besides, the citral chemotype partially stimulated the production of nitric oxide. The authors suggest that this work validates the essential oil efficacy based on the two *L. alba* chemotypes (citral and linalool) for the treatment of spasmodic visceral diseases, providing an alternative to *L. alba* infusions or its tinctures. (Blanco *et al.*, 2013).

In another study, the chemical composition and the pharmacological effects of two chemotypes of *L. alba* essential oil were evaluated: "citral" (COE) and "linalool" (LOE), in isolated ileum and duodenum of rats. COE had limonene, neral, geraniol and (-) – carvone as major components, whereas LOE presented linalool as its major component. The contractile curves of concentration-response (CRC) regarding acetylcholine (ACh) and K^+ 40 mM calcium (Ca^{2+} -CRC) were conducted on isolated intestinal portions, in the absence and presence of COE or LOE, both in different amounts. The results showed that verapamil, COE and LOE induced an ACh-CRC non-competitive inhibition, with an IC_{50} of $7,0 \pm 0,3$ mg COE/mL and $37,2 \pm 4,2$ mg LOE/mL. O 1-NAME, a NO-synthase blocker, increased the IC_{50} 's COE to $26,1 \pm 8,7$ mg COE/mL. In the same way, verapamil, COE and LOE did not inhibit competitively Ca^{2+} -CRC, with a CI_{50} of $6,3 \pm 1,7$ mg COE/mL, $7,0 \pm 2,5$ mg LOE/mL and $0,24 \pm 0,04$ mg verapamil/mL (pIC_{50} : 6,28). In this sense, it became clear that COE has five times higher potential than LOE to inhibit the muscarinic contractile responses. The essential oils from both chemotypes interfered in Ca^{2+} flux, although with the IC_{50} nearly 28 times higher than verapamil. In addition, the COE partially stimulated the NO production. These results prove the medicinal value of both *L. alba* chemotypes, thus validating its traditional usage, pharmacological potency, and mechanism of action (Blanco *et al.*, 2013).

In a work conducted by Carvalho *et al.* (2018), it was evaluated the effect of the *L. alba* essential oil (LaEO) composed by citral 75.92%

[geraniol (41.81%) and neral (34.11%)], limonene (9.85%), carvone (8.92%), gamma-terpinene (2.05%), cymene (1.02%) and its major constituents citral and limonene, isolated, on the tracheal smooth muscle of Wistar rats. The results demonstrated that LaEO, citral, and limonene promoted tracheal smooth muscle relaxation in the potassium-induced contractions (60 mM), presenting an EC_{50} of 148 ± 7 $\mu\text{g/mL}$ for LaEO, 136 ± 7 $\mu\text{g/mL}$ for citral and 581 ± 7 $\mu\text{g/mL}$ for limonene. In the acetylcholine-induced contractions (ACh; 10 μM), the CE_{50} for LaEO and citral was, respectively, 731 ± 5 $\mu\text{g/mL}$ and 795 ± 9 $\mu\text{g/mL}$. In preincubated preparations with 1000 $\mu\text{g/mL}$ of LaEO and citral, both agents blocked the influx of BaCl_2 by VOCCs. These results suggest that LaEO and citral have low activity in the muscarinic receptors and, thus, promote little or none Ca^{2+} influx through the calcium channels depending on the binder (SOCCs and ROCCs), since they require secondary signaling messengers (IP_3) and diacylglycerol (DAG), activated by the active ACh. The inhibition of the contractions caused by BaCl_2 suggests an interaction between the LaEO and citral with the VOCC receptors, which mediates the Ca^{2+} influx through the channel activation, due to the changes on the membrane potential. This study demonstrated that the LaEO and its main citral component presents antispasmodic effect on the tracheal smooth muscle of rats and the limonene has less potential than LaEO and citral, and it does not act through the cationic Ca^{2+} influx voltage-operated through the channels (Carvalho *et al.*, 2018).

Antimicrobial, antifungal and antiviral activities

Some recent researches highlight that the essential oil of *L. alba* in its citral chemotype is an alternative to synthetic fungicides and it can be used in organic agriculture (Glamoclija *et al.*, 2011). Costa *et al.* (2014) confirmed the activity of *L. alba* EO in its linalool chemotype against dermatophyte fungi.

The antifungal activity in two essential oils of *L. alba*, citral and carvone chemotypes, were evaluated against *Candida parapsilosis*, *Candida krusei*, *Aspergillus flavus* and *Aspergillus fumigatus*. The results showed that the most active essential oil was the citral chemotype, with minimal inhibitory concentration (MIC) of 78,7 and 270, 8 ng/mL for, respectively, *A. fumigatus* and *C. krusei*. The isolated citral substance, which is available commercially, showed an antifungal activity similar to the essential

oil citral chemotype (MIC values being 62,5 ng/mL to *A.fumigatus* and 39,7 µg/mL to *C. krusei*). The *L. alba* EO citral chemotype antifungal activity demonstrated in this study can be explained by a large concentration of oxygenated monoterpenes, such as neral/geranial (54%) and nerol/geraniol (8,9%) (Mesa-Arango, 2009).

In another study, the essential oil of *L. alba* – citral or neral chemotype – presented strong antimicrobial activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* (Barbosa, 2003).

L. alba antiviral activity was evaluated *in vitro* against the Human Herpesvirus-1 (HSV-1) in twenty EO samples collected in different regions across Colombia. In the collected essential oil samples, two chemotypes were identified: citral and carvone. The essential oils classified as “citral” chemotype (a mixture of neral and geranial isomers) present citral content (42–56%), followed by geraniol (7-16%) and trans-β-caryophyllene; the oils classified as chemotype “carvone” present carvone content (34–39%), followed by limonene (22–31%) beta-sesquifelandren (5–13%) and piperitenone (5–6%). The results demonstrated that the citral chemotype oils, as well as isolated major components, do not present activity against herpes simplex virus 1 in the tested amounts, which included cytotoxic and non-cytotoxic concentrations. However, the carvone chemotype showed moderate antiherpetic activity *in vitro*, in monolayers of infected HeLa cells, with values of Rf as $1 \times 10^{1.5}$ in concentrations from 125 µg/mL to 250 µg/mL. The positive controls, heparin sulfate, and acyclovir reduced the virus titer with Rf values to the order of 1×10^2 and 1×10^4 . None of the isolated monoterpenes showed activity against the HSV-1 (Agudelo-Gomez *et al.*, 2010).

Leishmanicidal action

Regarding the antileishmanial activity, the citral chemotype essential oil of *L. alba* was active against the extracellular forms of *Leishmania Viannia braziliensis*, in addition to the activity against the intracellular parasite form, being 2,8 times more selective to the parasite than the cells. The amphotericin B showed higher antiparasitic activity against the intracellular amastigotes of *Leishmania Viannia braziliensis* than against *Leishmania Viannia panamensis*, with an IS of 196 and 32, respectively. The essential oil displayed additional toxic effects in

THP-1 cells (Neira *et al.*, 2018).

Repellent and toxic activity for insects

The acaricide activity of *L. alba* essential oil, citral chemotype (44-46% geranial, 31-33% neral) and carvone chemotype (35-63% carvone and 25-27% limonene) were evaluated against the *Rhipicephalus microplus* tick. The acaricide efficacy was evaluated by the larval and immersion test in engorged females. The citral chemotype presented a higher larvicidal activity than the carvone chemotype, corroborating with the results obtained to the isolated citral, which presented larvicidal and adulticide activity with CL₅₀ values of 7,0 and 29,8 mg/mL, respectively. The isolated carvone showed a higher larvicidal activity than limonene, with R - (+) displaying a significant higher efficacy (CL₅₀ values of 31,2 mg/mL) than S - (-) (CL₅₀ values of 54,5 mg / mL). For the test with engorged female adults, both the essential oils and their isolated major components were less toxic, with the exception of citral, and this may be due to the limited cuticular penetration. Therefore, in this study, the essential oil citral chemotype presented a higher larvicidal efficacy than the carvone chemotype. The authors suggest that these results represent a green alternative to the tick control in bovines (Peixoto *et al.*, 2015a).

The repellent and toxic activity were evaluated against *Sitophilus zeamais* and *Tribolium castaneum*, which are insects that cause the loss of stored grains and considered as a widely distributed pest and global significance. Essential oils from different chemotypes of *L. alba* (carvone chemotypes LA-13 and LA-57 and citral chemotypes LA-10 and LA-44) and their main monoterpenes, carvone and citral, were evaluated. The bioassays of toxicity exposure of insects in treated filter paper were performed to determine the concentration and the lethal time. Repellency tests were conducted utilizing the most toxic compounds according to the toxicity bioassays. The carvone chemotypes were more toxic than the citral chemotype for two species: for the *S. zeamais* species, the CL₅₀ values were 15,2 µL/mL (LA-13) and 16,7 µL/mL (LA-57) and for the *T. castaneum* species, the LC₅₀ values were 28,7 µL/mL (LA-13) and 19,7 µL/mL (LA-57). The isolated carvone (CL₅₀ = 8,8 µL/mL) was more toxic than the citral. For the *S. zeamais*, the citral monoterpene presented the shortest lethal time (LT₅₀ = 6 h), whereas, for the *T. castaneum*, the carvone and citral

monoterpenes presented faster toxicity ($LT_{50} = 7,3$ h). The tested compounds were highly repellent to the *T. castaneum*; however, no repellent effect was observed against the *S. zeamais*, except for the LA-13 chemotype. The authors suggest that the *L. alba* carvone chemotype oils, in addition to the monoterpene carvone isolated components, have the potential for the development of natural insecticides against the *S. zeamais* and *T. castaneum* insects (Peixoto *et al.*, 2015b).

Cytotoxic and mutagenic activities

Two essential oils of *L. alba*, citral, and carvone chemotypes, were evaluated for their toxicity in Vero and HeLa cells culture. The essential oil of citral chemotype, containing 23,6% of neral and 30,5% of geraniol, demonstrated higher cytotoxic activity in the tumoral line HeLa and the lower activity in the cellular line Vero. The carvone chemotype essential oil did not demonstrate a cytotoxic effect. The absence of cytotoxicity in the carvone chemotype can be explained by the low content of citral oxygenated monoterpene (20,8%) compared to the citral chemotype (54,1%) or by the fact the major compounds (limonene and carvone) in the carvone chemotype essential oil are antagonists to the citral oxygenated monoterpene. The citral chemotype was not cytotoxic in non-tumoral Vero cells (Mesa-Arango, 2009).

Genotoxicity

The essential oil genotoxic properties of two chemotypes of *L. alba* collected in Bucaramanga (Colombia) were evaluated. The citral chemotype (geraniol 33,1%, neral 25,4%, trans beta caryophyllene 6,6%) and the carvone-limonene chemotype (carvone 38,1%, limonene 33,2%, beta-sesquiphellandrene 6%) did not present genotoxicity, although one of the isolated components – limonene – presented genotoxicity in doses between 97 and 1549 mM. Both chemotypes protected bacterial cells against the bleomycin-induced genotoxicity. Antigenotoxicity in two chemotypes of *L. alba* seems to be linked to citral and carvone compounds respectively. (López *et al.*, 2011).

On the other hand, the citral chemotype essential oil of *L. alba* collected in Colombia, being used as a treatment of 100 mg/kg for 14 days (orally) did not induce physical changes such as

weight loss or pain signal increase in the treated animals. However, this treatment induced a slight damage to the DNA, with damage values around 23%. The induced damage by the cyclophosphamide positive control (CPA) was three times higher ($74,9 \pm 3,2\%$) with significant differences ($p < 0,001$) between them. The essential oil did not display the formation of micronuclei in erythrocytes of polychromatic bone marrow. By contrast, the genotoxicity positive control CPA showed the frequency of $175 \pm 18,8$ MN in 1000 polychromatic erythrocytes (Neira *et al.*, 2018).

Antioxidant and adaptogenic activity

The effects of the addition of essential oil of *L. alba* of linalool chemotype in the diet of silver catfish were evaluated. These effects were evaluated following blood, metabolic, growth and oxidative stress biomarkers parameters. The essential oil evaluated in this study presented, as major compounds, linalool (55.26%), 1,8-cineole (7.85%), and γ -muurolene (4.63%). The results demonstrated the diet containing essential oil of *L. alba* did not influence in the blood and growth and blood parameters of these animals. However, the diet decreased the glucose levels and increased liver glycogen stores. The amount of essential oil, in the ratio of 1,0 ml per kilogram of feed, increased the lactate levels in the liver; 0,5 mL/kg increased the glycogen levels in the muscle; the antioxidant response of tissues was also increased by the essential oil diet. The authors suggest the essential oil of *L. alba* may have caused the sedation and decreased metabolism, thus increasing both liver and muscle glycogen stores and the antioxidant responses in different tissues as well, a mechanism demonstrating the theory of adaptation (Saccol *et al.*, 2013).

Puerta-Meija *et al.*, (2002) demonstrated low antioxidant activity in the essential oil from Colombia, having carvone (61%) as the major compound (Puertas- Mejía *et al.*, 2002). Another study performed by Stashenko *et al.* (2003) highlighted visible antioxidant activity, comparable to vitamin E. The major compounds from this study were carvone and limonene, suggesting that the antioxidant action could be attributed to the presence of limonene (Stashenko *et al.*, 2003).

Activities in the Cardiovascular System

Maynard *et al.* (2011) investigated the essential oil of *L. alba* effect containing, as major constituents, geranial (48,58%) and neral (35,42%) on the mesenteric artery of rats. The results demonstrated an endothelium-independent vasorelaxation, which is caused, seemingly and partially, by the calcium influx blockage, mediated by the voltage-operated calcium channels.

Clinical Trial for patients with headaches (migraines)

It was realized a phase II cohort, non-controlled and prospective clinical trial to evaluate the therapeutic effects of *L. alba* on patients with migraines. The patients received hydroalcoholic extracts from leaves. The intensity and frequency of the symptoms were monitored before and after 30-60 days of treatment. The study demonstrated that the chemotype containing carvone and geraniol as its major compounds was able to significantly reduce the frequency and intensity in the pain episodes. Over 80% of the volunteers experienced a minimal reduction of 50% in pain frequency and intensity, with no observed side effects (Conde *et al.*, 2011).

Non-clinical toxicity evaluation: Dermal toxicity

The citral chemotype EO of *L. alba*, when submitted to dermal toxicity evaluation assay (OECD method, 2015) did not present toxicity to 10, 50 and 100% concentrations, being considered as non-irritating (Neira *et al.*, 2018).

Acute oral toxicity

The evaluation for citral chemotype EO of *L. alba* acute oral toxicity was conducted utilizing the maximum oral dose of 2000 mg/kg. The results demonstrated the animals treated with citral chemotype EO of *L. alba* did not show clinical signs of systemic toxicity, with a survival rate of 100%. Major statistic differences in the weight of the animals before and after the treatment (weight of

$22,47 \pm 0,9$ g versus $22,74 \pm 1,0$ g) were not observed, as well as pain signs or behavior change during the days of treatment. The weight and daily consumption of food happened as expected, exhibiting well-being. The macroscopic analysis of the organs did not show any changes, thus presenting a normal aspect. (Neira *et al.*, 2018).

In another study, mice treated orally with a single dose (1500 mg/kg) of citral chemotype essential oil of *L. alba* (geranial 45,3%, neral 30,23% and beta-farnesene 7,78%) presented a survival rate around 60%. Lowering the dose (300 and 900 mg / kg) there was a 100% survival rate, with slight signs of ataxia, lethargy, salivation and transitory seizures. The effect produced by this chemotype, according to the authors, is related to the action that citral has on the central nervous system, due to the inhibitory activity of citral on the synthesis of retinoic acid, an essential metabolite for the neuronal differentiation and development (Zhang *et al.*, 2009; Aular *et al.*, 2016).

The Table N° 1 shows ten chemotypes quoted in the publications and their different biological activities. Table N° 2 presents the pharmacological actions with its respective isolated substances from the essential oils of *L. alba*.

CONCLUSION

The studies indicate the carvone, citral-limonene, and citral chemotypes present pharmacological depressant activity in the central nervous system, acting on anxiety control. These studies corroborate with the traditional usage of *Lippia alba*, which is designated as tranquilizer, sedative, anxiolytic.

The broad array of pharmacological activities presented by the several chemotypes in the essential oil of *L. alba* demonstrate the importance of the chemical monitoring of the species for the definition of the clinical application of essential oil. In this sense, defining the chemotype and monitoring the content of its constituents is paramount for the development of drugs from this species.

Table N° 1
Pharmacological effects of different chemotypes of *Lippia alba*

| Essential oil Chemotype | Pharmacological Activity | Reference |
|---------------------------|--|--|
| Carvone | Anxiolytic | Vale, 1999 |
| | Anti-herpetic | Agudelo-Gomez <i>et al.</i> , 2010 |
| | Antigenotoxic | Lopez <i>et al.</i> , 2011 |
| | Repellant and Toxic against <i>Sitophilus zeamais</i> and <i>Tribolium castaneum</i> | Peixoto <i>et al.</i> , 2015b |
| Citral-limonene | Anxiolytic, sedative, hypothermic and myorelaxant | Vale, 1999 |
| | Anti-inflammatory, antiedematogenic, analgesic | Viana <i>et al.</i> , 1998 |
| Citral | Moderate action on the benzodiazepine receptors | Hennebelle <i>et al.</i> , 2008 |
| | Cytotoxic in the tumoral line | Mesa-Arango, 2009 |
| | Anticonvulsant | Viana <i>et al.</i> , 2000 |
| | Antispasmodic | Blanco <i>et al.</i> , 2013, Carvalho <i>et al.</i> , 2018 |
| | Antifungal | Glamočlija <i>et al.</i> , 2011, Mesa-Arango, 2009 |
| | Antimicrobial | Barbosa, 2003 |
| | Acaricide | Peixoto <i>et al.</i> , 2015a |
| | Leishmanicidal | Neira <i>et al.</i> , 2018 |
| | Antigenotoxic | Lopez <i>et al.</i> , 2011 |
| | Vasorelaxant | Maynard <i>et al.</i> , 2011 |
| | Anesthetic | Batista <i>et al.</i> , 2018 |
| Myrcene | Anticonvulsant | Viana <i>et al.</i> , 2000 |
| Limonene | Anticonvulsant | Viana <i>et al.</i> , 2000 |
| | Antispasmodic | Carvalho <i>et al.</i> , 2018 |
| Limonene-carvone | Mucolytic | Matos, 2007 |
| Carvone-limonene | Anti-inflammatory, antiedematogenic | Viana <i>et al.</i> , 1998 |
| | Antioxidant | Stashenko <i>et al.</i> , 2003 |
| Myrcene-citral | Antispasmodic | Matos, 2007 |
| Linanool | Antifungal | Costa <i>et al.</i> , 2014 |
| | Antispasmodic | Blanco <i>et al.</i> , 2003 |
| | Antioxidant | Saccol <i>et al.</i> , 2013 |
| | Adaptogenic | Saccol <i>et al.</i> , 2013 |
| Carvone-citral (geraniol) | Analgesic | Conde <i>et al.</i> , 2011 |

Table N° 2
Pharmacological effects of major constituents in the essential oil of *L. alba* species

| Major Constituents | Pharmacological Effects | Reference |
|--------------------|--|------------------------------|
| Citral | Sedative and myorelaxant, in addition to the pentobarbital potentiation on rodents | Vale <i>et al.</i> , 2009 |
| | Anticonvulsant | Barbosa, 2003 |
| | Gastroprotective activity | Ortiz, <i>et al.</i> , 2010 |
| Myrcene | Sedative and myorelaxant, in addition to the pentobarbital potentiation on rodents | Vale <i>et al.</i> , 2009 |
| | Anticonvulsant | Barbosa, 2003 |
| Limonene | Sedative and myorelaxant, in addition to the pentobarbital potentiation on rodents | Vale <i>et al.</i> , 2009 |
| | Anticonvulsant | Barbosa, 2003 |
| | Gastroprotective | Moraes, <i>et al.</i> , 2009 |
| 1,8 cineol | Gastroprotective | Santos & Rao, 2001 |
| Linalool | Gastroprotective | Barocelli et al., 2004 |
| | | |

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