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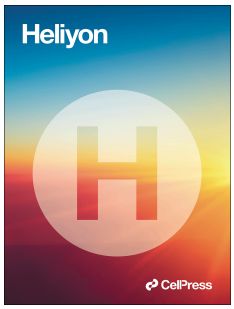


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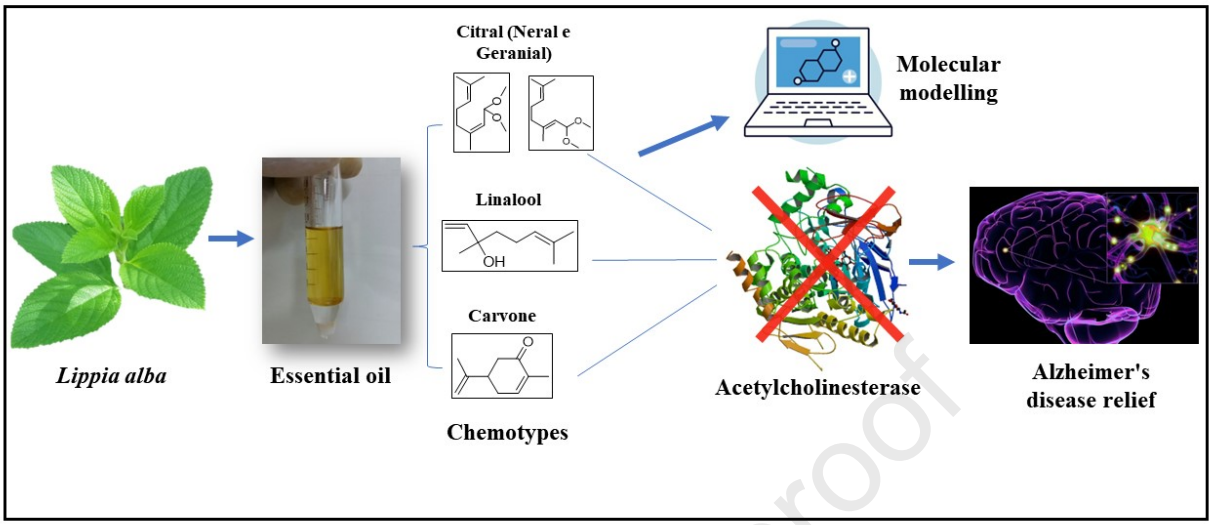

# Molecular modelling and anticholinesterase activity of the essential oil from three chemotypes of *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae)

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# Molecular modelling and anticholinesterase activity of the essential oil from three chemotypes of *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson (Verbenaceae)

**Abstract**

*Lippia alba* (Mill.) N.E. Brown (Verbenaceae), popularly known as "erva cidreira", is one of the most used plants in Brazilian folk medicine. The species has several chemotypes and its volatile constituents have already been characterized, and present different chemical markers with known pharmacological properties, such as analgesic, sedative and antifungal properties. The objective of this study was to evaluate the anticholinesterase activity (AChE) of the essential oil of three chemotypes of *Lippia alba* and, by using molecular anchoring, determine the best receptor-ligand interaction energies of the main constituents present in the samples of oil. The essential oils were obtained via hydrodistillation (LA1 and LA2) and steam drag (LA3), and their volatile constituents determined using GC-MS. For the determination of anticholinesterase activity, direct bioautography and colorimetry assays based on Ellman's method were used. Molecular docking was performed using a multiple solution genetic algorithm and Merck molecular force field 94 (MMFF94) as the scoring function. In the main constituents of the oil samples, three chemotypes were identified for *L. alba*: LA1 is rich in citral, LA2 is rich in carvone and LA3 is rich in linalool. All *L. alba* chemotypes showed AChE enzyme inhibition with an $IC_{50}$ of 3.57 µg/mL (LA1), 0.1 µg/mL (LA2) and 4.34 µg/mL (LA3). The molecular docking study complemented the results of the experiment and demonstrated significant interactions between the main constituents of the oils and the amino acid residues of the AChE enzyme. Irrespective of the chemotype, *Lippia alba* presents biotechnological potential for the discovery of anticholinesterase substances, with the chemotype LA2 (rich in carvone) being the most active.

**Keywords:** erva cidreira; aromatic plant; acetylcholinesterase inhibitors.

## 1. Introduction

*Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson, popularly known as lemon balm and/or false melissa, is an aromatic plant that belongs to the Verbenaceae family and is widely distributed in several tropical and subtropical regions of the Americas. Since ancient times, traditional communities have used this plant species in their daily lives, giving it a significant relevance in historical, cultural and medicinal contexts [1]. Generally, *L. alba* is cultivated in domestic environments and is traditionally prepared as an infusion, maceration, decoction, and used in compresses, baths or extracts to relieve stress, insomnia and the symptoms of flu and colds [2]. Other uses also include cases of diarrhea, cramps, bronchitis, hypertension, headache and liver disorders [3,4].

Many studies have attributed pharmacological activities to the essential oil of this plant such as antibacterial [5, 6], anesthetic [7] antiparasitic [8], antiviral [9], antioxidant, sedative/relaxing [10, 11], antispasmodic [12], and anxiolytic [13] activities, in addition to efficacy in aromatherapy to reduce psychological stress [14]. The essential oil of *L. alba* is composed of a variety of substances, being citral (mixture of geranial and neral isomers) one of the main components responsible for its characteristic aroma; however, other compounds may be present in the composition of the essential oil, since the same species can present different chemical types (chemotype).

These quantitative and qualitative variations in the chemical composition of *L. alba* essential oil have been widely proven, and have led to the classification of the species into different chemotypes according to its main constituents [15], the most described in the literature being citral, carvone and linalool (Figure 1). However, 1,8-cineole, myrcene and β-elemene also occur [16, 17, 18]. In the Amazon, there is a report of the chemotypes citral [6], carvone-limonene [19, 20], myrcene-citral [21], citral-carvone-limonene [22], limonene-1,8-cineole, carvone-limonene-germacrene D, and citral-germacrene D [16].

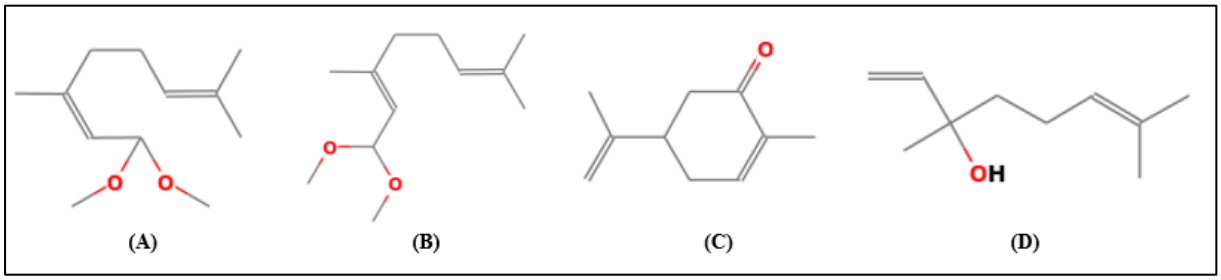



**Figure 1** - Chemical structure of the majority components of the main chemotypes of *L. alba*. (A) = citral - neral dimethyl acetal; (B) citral - geranial dimethyl acetal; (C) = carvone and (D) = linalool. Source: NIST.

The occurrence of chemotypes in this species have aroused the interest of the academic community since, depending on the chemical composition, the biological activity of a plant species can undergo considerable changes. Therefore, it is important to evaluate the effect of different chemotypes of *L. alba* in different biological activities. As the species has been used by communities without regards to its chemical composition, this has led to inappropriate use of the species. In Table 1, it is possible to observe the studies that have been carried out with the different chemotypes of *L. alba.*

In this brief review (Table 1) of studies involving *L. alba* essential oil that have been carried out in the last five years (2020-2024) and are available on the PubMed search platform, it can be noted that there is a lack of studies that prove the action of this plant on the central nervous system (CNS). Many diseases that affect the CNS have no cure and, among the pathologies that affect the CNS, Alzheimer's disease (AD) stands out. Its treatment is based on the use of drugs that inhibit the enzyme acetylcholinesterase (AChE), which is an important natural organic substance responsible for hydrolyzing the neurotransmitter acetylcholine, and is often used in prospective studies of plant species with therapeutic action for neurodegenerative pathologies such as AD [23].

Thus, since comparative studies between the different chemotypes of *L. alba* are still incipient, the objective of this study was to evaluate whether the essential oil of three chemotypes of *Lippia alba* have an inhibitory effect on the enzyme acetylcholinesterase. Currently, a gap remains in the literature regarding the use of the different chemotypes of *L. alba* as acetylcholinesterase inhibitors or for other pharmacological activities. As the species is already used by the population, mainly as an alternative treatment for various types of diseases and without chemotype discrimination, this study shows that the inhibitory effect of *L. alba* essential oil on the enzyme acetylcholinesterase is dependent on the chemical constitution of the chemotype used.

Since it is an aromatic plant with a high essential oil content, *L. alba* can be used in aromatherapy or for the development of more sustainable chemical products, thus reducing environmental impacts and saving materials. Therefore, the use of essential oils for the development of phytoproducts favors green chemistry, a segment of chemistry that seeks alternative processes that generate less pollution and less waste, and presents high energy efficiency, in addition to valuing the use of renewable raw materials and generating biodegradable and safe products for society and the environment.

In addition to knowledge about the activity of the chemical constituents of *L. alba* in the inhibition of acetylcholinesterase, it is important to understand its mechanism of action and its pharmacokinetic properties. Thus, in the present work, we also conducted a study on the interaction between the main compounds of each oil and the human acetylcholinesterase enzyme, as well as a detailed *in silico* pharmacokinetic study in order to evaluate the probability of these compounds being absorbed orally and reaching the central nervous system to finally interact with the molecular target and present an effective pharmacological response.

122 **Table 1:** Main studies that evaluated the biological activities of the essential oil from different chemotypes of *L. alba*, published in the period
123 2020-2024.

| Number | Author(s) | Title | Chemotype(s) | Biological activity evaluated | Year | Reference |
|---|---|---|---|---|---|---|
| 1 | Barbosa et al. | *In vitro* anthelmintic activity of *Lippia alba* essential oil chemotypes against *Haemonchus contortus*. | Citral and carvone | Anthelminth | 2023 | [24] |
| 2 | Bonilla-Carvajal et al. | Essential Oil of Carvone Chemotype *Lippia alba* (Verbenaceae) Regulates Lipid Mobilization and Adipogenesis in Adipocytes. | Carvone | Lipid mobilization and adipogenesis | 2022 | [25] |
| 3 | Quintero et al. | Immunomodulatory, trypanocide, and antioxidant properties of essential oil fractions of *Lippia alba* (Verbenaceae). | Citral and carvone | Trypanocide and immunomodulator | 2021 | [26] |
| 4 | Lima et al. | Insecticidal activity of a chemotype VI essential oil from *Lippia alba* leaves collected at Caatinga and the major compound (1,8-cineole) against *Nasutitermes corniger* and *Sitophilus zeamais.* | 1,8-cineol | Insecticide | 2021 | [27] |
| 5 | Borges et al. | Effect of *Lippia alba* (Mill.) N.E. Brown Essential Oil on the Human Umbilical Artery. | Citral | Vasorelaxant in human umbilical arteries (HUA) | 2022 | [28] |

124 **Table 1**: (Continued)

| Number | Author(s) | Title | Chemotype(s) | Biological activity evaluated | Year | Reference |
|---|---|---|---|---|---|---|
| 6 | | Ovicidal effect of essential oils of *Lippia alba*, *Lippia sidoides* and *Lippia gracilis* on the acanthocephalan *Neoechinorhynchus buttnerae* (Eoacanthocephala: Neoechinorhynchidae). | | Ovicide (*Neoechinornchus buttnerae*) | 2022 | [29] |
| 7 | Postay et al. | The effectiveness of surfactants applied with essential oil of *Lippia alba* in the anesthesia of Nile tilapia (*Oreochromis niloticus*) and their toxicity assessment for fish and mammals. | Linalool | Anesthetic/Toxicity | 2021 | [30] |
| 8 | Gomes et al. | *Lippia alba* and *Lippia gracilis* essential oils affect the viability and oviposition of *Schistosoma mansoni.* | Citral | Anthelminth | 2022 | [31] |
| 9 | Filho et al. | Chemical composition and biological activities of the essential oils from *Lippia alba* and *Lippia origanoides.* | Citral/Limonene | Antioxidant; antimicrobial and acute toxicity | 2023 | [32] |
| 10 | de Lima et al. | Eugenol and *Lippia alba* essential oils as effective anesthetics for the Amazonian freshwater stingray *Potamotrygon wallacei* (Chondrichthyes, Potamotrygonidae). | Eugenol | Anesthetic | 2021 | [33] |

125 **Table 1**: (continued)

| Number | Author(s) | Title | Chemotype(s) | Biological activity evaluated | Year | Reference |
|---|---|---|---|---|---|---|
| 11 | Pagotti et al. | Trypanocidal Activity of *Dysphania ambrosioides*, *Lippia alba*, and *Tetradenia riparia* Essential Oils against *Trypanosoma cruzi.* | Linalool | Trypanocide | 2021 | [34] |
| 12 | Nonato et al. | Comparative analysis of chemical profiles and antioxidant activities of essential oils obtained from species of *Lippia* L. by chemometrics. | Citral | Antioxidant | 2022 | [35] |
| 13 | de Brito et al. | Identification of Bioactive Compounds against *Aedes aegypti* (Diptera: Culicidae) by Bioassays and in Silico Assays. | Citral | Repellent | 2021 | [36] |
| 14 | Tabari et al. | Acaricidal activity, mode of action, and persistent efficacy of selected essential oils on the poultry red mite (*Dermanyssus gallinae*). | Carvone-Limonene | Acaricide | 2020 | [37] |

126 Source: Authors (2024).

## 2. Materials and Methods

### 2.1 Plant Material

The aerial parts (leaves, flowers and thin branches) of two specimens of *L. alba* (Mill.) N.E.Br. ex Britton & P. Wilson (named LA1 and LA2) were collected in the village of Alter do Chão (2º30'31.0" S and 54º57'00.0" W), Santarém, Pará, Brazil, in the months of May and July 2021. Of these, exsiccates were prepared and deposited in the herbarium of the University of Juiz de Fora, Minas Gerais, under the number CESJ 65276, and the taxonomic confirmation was carried out by the specialist in Verbenaceae, Dr. Fátima Salimena. This research was registered in SisGen (Sistema Nacional de Gestão do. Patrimônio Genético e do Conhecimento Tradicional Associado) under the number A965D42.

The third sample of *L. alba* essential oil (LA3) was obtained from a commercial crop in Serra Negra, São Paulo (22°31'33.4" S 46°42'10.8" W) that was produced by DJUH Indústria e Comércio de Cosméticos Ltda. (batch: OMLA-0001/18), and was provided by Dr. Pedro Melillo Magalhães.

### 2.2 Acquisition of the essential oils

*L. alba* leaves (LA1 and LA2) were previously dehydrated in a forced air circulation oven at a temperature of 37 ± 2 °C and then subsequently subjected to the hydrodistillation process using a Clevenger-type apparatus for 120 min. The LA3 oil sample was obtained from dehydrated aerial parts via steam distillation for 3 hours.

All the oil samples were subjected to centrifugation with anhydrous sodium sulfate to remove water. After centrifugation, the oil was removed with a pipette and stored in an amber bottle, hermetically closed, and stored in a refrigerator at 5 ºC until analysis. The yield was calculated based on the dry weight of the plant [38].

### 2.3 Analysis of the volatile constituents of the samples

The samples of the essential oil were analyzed in a gas chromatography system coupled to a mass spectrometer (GCMS-QP2010 Ultra, Shimadzu Corporation, Tokyo, Japan), equipped with an auto injector (AOC-20i) and CGMSsolution software, which contains databases of libraries [39] including FFNSC 2 [28] and a fused silica capillary column (Rxi-5ms, Restek Corporation, Bellefonte, PA, USA) of 30 m x 0.25 mm (diameter) x 0.25 µm (film thickness), coated with 5% diphenyl dimethylpolysiloxane. The analysis conditions were as follows: helium drag gas (99.995%); split ratio mode in the ratio of 1:20; injection of 1 µL of the sample (3 µL of the essential oil in 500 µL of hexane); ionization

energy by electronic impact (EI) 70 eV; injector temperature: 250 ºC; oven temperature program: 60-240 ºC; ion source temperature: 200 ºC; transfer line temperature: 250 ºC.

Quantitative data on the volatile constituents were obtained via peak area normalization using a gas chromatograph (GC 6890 Plus series, Agilent) coupled to a flame ionization detector (FID), which was operated under similar conditions to the GC-MS system. The mass spectra were obtained by automatic scanning at 0.3 scans/second, with mass fragments of 35-400 *m/z*. The compounds found in the ion chromatograms were identified by comparing the mass spectra (molecular mass and fragmentation pattern) with those found in the system's CGMSsolution library and by comparison with the retention indexes. The linear equation of Van den Dool and Kratz (1963) [40] was used to calculate the volatile components, with the use of a standard homologous series of C8-C20 n-alkanes (Sigma-Aldrich).

### 2.4 Determination of the cholinesterase inhibition

For the *in vitro* anticholinesterase assays, the enzyme acetylcholinesterase type VI-S, obtained from *Electrophorus electricus* (lyophilized powder, C3389-2Ku, Sigma-Aldrich, batch: SLBZ8573) was used. The standard used in the assays was the anticholinesterase inhibitor eserine (physostigmine) (Sigma-Aldrich, batch: BCBC4171V) diluted in methanol. A standard curve was used to define the concentration used in the tests.

#### 2.4.1 Qualitative test on a thin layer chromatography (TLC) chromatoplate

For the qualitative testing of the samples of *L. alba* essential oil and the standard drug, an aluminum chromatoplate for TLC (ALUGRAM® Xtra SIL G, silica gel 60, 0.20 mm, Macherey-Nagel) was used based on the direct bioautography method of Marston et al. (2002) [41]. The enzyme was diluted in tris-HCl buffer, 50 mM, pH 7.8 in ultra-pure water to obtain a concentration of 4 U/mL, with the addition of bovine serum albumin (Sigma-Aldrich) at a ratio of 1:1. Samples of *L. alba* essential oil were diluted in methanol at a concentration of 100 µL/mL. Physostigmine at a concentration of 100 µg/mL was used as the standard. For the negative control, methanol was used. The colorimetric reagents of the test were naphthyl acetate (2.5 mg in methanol) and Fast Blue B salt (2.5 mg in ultrapure water). Both reagents were prepared and mixed immediately before use to prevent their decomposition.

To perform the test, the aliquots of 10 µL of the oil samples and controls were applied to the chromatoplates in duplicate, and allowed to stand for a period of 24 h for evaporation of the solvent. Subsequently, the plate was sprayed with the acetylcholinesterase enzyme

191 solution (4 U/mL) and incubated in a humidity test chamber, without direct contact with
192 moisture, at 37 °C for 20 minutes. Then, the plate was sprayed with the mixture of naphthyl
193 acetate (2.5 mg) and Fast Blue B salt (2.5 mg) solutions to obtain the final results. The
194 formation of a purple coloration occurred gradually, after 1 to 3 min.

195 2.4.2 Quantitative assay

196 The assay for the quantification of acetylcholinesterase inhibition of the samples was
197 adapted from Ellman's method [42], with modifications, as described by [43]. In summary,
198 three buffers were produced for the quantitative test, which were denominated A, B and C.
199 These being: buffer A = 50 mM Tris/HCl, pH 8, dissolved in ultrapure water; buffer B = 0.1%
200 bovine serum albumin in buffer A; and buffer C = 0.1 M NaCl and 0.02 M $MgCl_2.6H_2O$
201 dissolved in buffer A.

202 In a total volume of 1 mL, 415 µL of buffer A, 10 µL of the essential oil solution
203 (diluted in methanol, buffer and Tween 80) at different concentrations (100, 50, 25, 12.5, 6.25
204 and 3.12 ug/mL), and 75 µL of acetylcholinesterase enzyme, containing 0.2 U/mL, were
205 added. The samples were then incubated for 15 min at 25 °C. After incubation, 75 µL of a
206 solution of 1.83 mM AChI (acetylthiocholine iodide) (Sigma-Aldrich, Steinheim, Germany)
207 and 425 µL of 3 mM DTNB (5,5'-dithiobis[2-nitrobenzoic acid]) (Sigma-Aldrich, Steinheim,
208 Germany) were added and the mixture was incubated for 30 min at 25 °C under a light source.
209 The absorbance of the mixture was measured at 412 nm in a UV spectrophotometer (NOVA,
210 3300). Physostigmine was used as the standard drug and a dilution solution was used as
211 negative control (buffer A, methanol and Tween 80 at a ratio of 2:2:1). The percentage of
212 inhibition of enzyme activity was calculated according to the equation % = [(A0 – A1) / A0] *
213 100, where A0 was the absorbance of the control without the essential oil and A1 was the
214 absorbance of the essential oil sample at different concentrations. All tests were performed in
215 triplicate. The concentration of the sample that provided 50% inhibition ($IC_{50}$) was obtained
216 by constructing graphs of the percentages of inhibition versus the concentration of the
217 inhibitor. The non-linear regression parameters for the curve were plotted and the $IC_{50}$ values
218 were obtained using the Microsoft Excel 2019 software.

219 2.4.3. Evaluation of the possible synergistic effect of the essential oils

220 To evaluate the possible synergistic effect of the different essential oil samples of the
221 chemotypes of *L. alba* on the acetylcholinesterase enzyme, mixtures of the three oil samples
222 in different proportions were made, according to Table 2. The $IC_{50}$ was determined using the

same conditions mentioned above. The chemical composition of the mixtures was also evaluated using GC-MS in order to confirm the presence of the main constituents after mixing.

**Table 2.** Mixtures obtained using essential oil of the three chemotypes of *Lippia alba.*

| Oil samples | | | |
|---|---|---|---|
| **LA1** | **LA2** | **LA3** | **Pool** |
| Proportion of essential oil in the mixture (μL) | | | |
| 100 | 100 | 100 | Pool 1 |
| 100 | 0 | 100 | Pool 2 |
| 100 | 100 | 0 | Pool 3 |
| 0 | 100 | 100 | Pool 4 |

Source: Authors (2024).

LA1 = *Lippia alba* citral chemotype; LA2 = *Lippia alba* carvona chemotype; LA3 = *Lippia alba* linalool chemotype.

## 2.5 Molecular modelling and ADMET properties

### 2.5.1 Preparation of the ligands

The chemical structures of the main compounds of *Lippia alba* were obtained from the National Institute of Standards and Technology (NIST, available at https://webbook.nist.gov/cgi/cbook.cgi?ID=R185885). Subsequently, the compounds underwent optimization of their three-dimensional structure in the software ChemSketch (available at www.acdlabs.com) using the molecular mechanics method. Subsequently, the 2D structures were optimized using ChemSketch (available at www.acdlabs.com), that contains a 3D optimization algorithm modified from a molecular mechanics package (CHARMM) that consider angle bending, bond stretching, internal rotation, and van der Waals non bonded interactions [44]. During the 3D optimization, the stereo bonds of compounds with well-defined stereochemistry were maintained and were not replaced by single bonds.

### 2.5.2 Preparation of the AChE crystal structure

The crystallographic structure of human acetylcholinesterase (*h*AChE) complexed with the inhibitor donepezil was obtained from the Protein Data Bank (PDB, available at https://www.rcsb.org/), under the PDB code 4EY7 [45]. Then, the crystallographic complex

was treated with the software BIOVIA Discovery Studio® v. 20.1.0, whereby the water molecules [46], the enzymatic co-factors and the co-crystallized ligand were removed, leaving only the chain of interest containing the binding site with donepezil.

### 2.5.3 Re-docking

In order to validate the molecular docking of the main compounds of the studied plant species, donepezil was anchored to the 4EY7 binding site through the DockThor web server (https://www.dockthor.lncc.br/v2/).

The molecular docking was performed using a multiple solution genetic algorithm and Merck molecular force field 94 (MMFF94) as the scoring function. The in-house program, PdbThorBox, is applied to set the protein atoms, the partial charges and complete missing side chains of the protein file [47], according to MMFF94 force field.

At this stage, the modules "Rotatable Bonds Enable all" and "Add Hydrogen Disabled" were used to prepare the binder on the server. Regarding anchoring, the grid box dimension was 20x20x20 Å and the pre-selected algorithm precision settings were conserved. After completing the molecular docking, the results were filtered by setting the modules "RMSD to cluster conformers" equal to 2 and "Number of binding modes" equal to 10.

The RMSD (root-mean-square deviation) value was calculated via the Discovery Studio Software. To analyze the docked pose, donepezil was used as a reference molecule. Via the "Structure" > "RMSD" > "Heavy Atoms" module, it was possible to obtain the RMSD value report, which was obtained from the comparison between the pose with the best score of re-docking and donepezil. Molecular docking parameterization was established based on an RMSD value of below 1.5 Å, which indicates that the molecular docking protocol can be used for docking of other ligands [48].

### 2.5.4 Molecular docking

The molecular docking of the main compounds of *Lippia alba* with a crystal structure (PDB: 4EY7) followed the same method of the re-docking step with the co-crystallized ligand, with exception of the RMSD calculation. At this step, physostigmine, a well-known cholinesterase inhibitor, was used as control of molecular docking study. The DockThor program generated ten energetically favorable conformations of the ligands in the active site of the enzyme. Via DockTScore, which is a linear empirical function coupled to the DockThor portal, binding affinities were predicted, with lower values indicating high binding

affinity for *h*AChE. The interpretation and visualization of the molecular interactions were performed using the Discovery Studio software.

2.5.5 Pharmacokinetic prediction

The screened phytochemicals were subjected to pharmacokinetic analysis using the online prediction tool Swiss ADME (available at http://www.swissadme.ch/). This program provides the physicochemical properties and ADME (absorption, distribution, metabolism, and excretion) parameters associated with the pharmacokinetics of each compound. Data calculated included the number of rotatable bonds (NRB), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), $LogP_{o/w}$ values as a measure of lipophilicity, gastrointestinal (GI) absorption, and blood brain barrier (BBB) permeation. SwissADME predicts human gastrointestinal absorption (HIA) and BBB through the BOILED-Egg model, a classification chart, which defines favorable and unfavorable zones in the physicochemical space of lipophilicity versus polar surface area, for passive diffusion through of the two physiological barriers [48].

2.6 Statistical analysis

Data from the quantitative anticholinesterase assay were analyzed using the Prism 5 software with one-way ANOVA, followed by the Tukey test with multiple comparisons, at a significance level of $p<0.05$. The GraphPad Prism 8.0.1 software was used for the statistical evaluation of binding affinity values through a one-Way ANOVA, which was followed by Sidak's multiple comparison test in order to evaluate and infer differences to the chosen pivot molecule.

## 3. Results

3.1 Volatile constituents of the essential oil from the three chemotypes of *Lippia alba*

The volatile constituents of the essential oils of *L. alba* samples and their quantities are presented Table 3. According to their main constituents, *L. alba* was classified into three distinct chemotypes: citral chemotype – LA1; carvone chemotype – LA2 and linalool chemotype – LA3. As its main constituents, LA1 presented neral (23.84%), geranial (32.31%), γ-muurolene (7.67%) and limonene (7.02%); LA2 presented carvone (30.72%), 1,8-cineole (14.37%) and limonene (10.3%), and LA3 presented as its main constituent linalool (68.31%). The essential oil content measured in g/g dry weight, which ranged from

309 1.1 to 3.7% according to the chemotype. Table 3 also shows the volatile constituents of the
310 different mixtures of the *L. alba* essential oil.

311 **Table 3 –** Volatile constituents and essential oil yield of three samples *Lippia alba* and their mixtures (pools).

| **Essential oils** | | | LA1 | LA2 | LA3 | Pool 1 | Pool 2 | Pool 3 | Pool 4 |
|---|---|---|---|---|---|---|---|---|---|
| **Yield** | | | 3.7% | 1.1% | 1.3%* | - | - | - | - |
| Constituents | $RI_{calc}$ | $RI_{lit}$ | | | | (%) | | | |
| α-Pinene (HM) | 932 | 932 | - | 0.56 | - | 0.19 | - | - | - |
| Sabinene (HM) | 972 | 969 | - | 3.74 | - | 1.48 | 0.86 | 3.02 | 3.31 |
| 6-methyl-5-Hepten-2-one (HM) | 983 | 983 | 1.9 | - | - | - | - | - | - |
| Mircene (HM) | 989 | 988 | 0.9 | 3.89 | - | 1.73 | - | 1.79 | 1.9 |
| δ-3-Carene (HM) | 1012 | 1008 | - | - | - | - | - | - | - |
| *p*-Cymene (HM) | 1023 | 1020 | 0.77 | - | - | - | - | 0.94 | - |
| **Limonene** (HM) | 1027 | 1024 | **7.02** | **10.3** | - | **6.11** | **0.94** | **7.43** | **5.88** |
| **1,8-Cineole** (OM) | 1030 | 1026 | - | **14.37** | - | **5.99** | - | **11.72** | **12.86** |
| *trans*-β-Ocimene (HM) | 1046 | 1044 | 0.53 | 0.96 | 1.98 | 0.61 | - | - | - |
| γ-Terpinene (HM) | 1057 | 1054 | 2.78 | - | - | 0.77 | - | - | - |
| *cis*-Sabinene hydrate (OM) | 1065 | 1065 | - | 0.52 | - | - | - | - | - |
| *cis*-Linalool oxide (OM) | 1071 | 1067 | - | - | 1.81 | - | 0.62 | - | 0.62 |
| *trans*-Linalool oxide (OM) | 1088 | 1084 | - | - | 1.58 | - | 0.56 | - | 0.59 |
| **Linalool** (OM) | 1104 | 1095 | 0.77 | 0.75 | **68.31** | **28.11** | **47.53** | **0.89** | **42.7** |
| *endo*-Fenchol (OM) | 1106 | 1114 | - | - | 2 | - | - | - | - |
| *trans*-Verbenol (OM) | 1143 | 1140 | - | 0.73 | - | - | - | - | - |
| δ-Terpineol (OM) | 1165 | 1162 | - | 0.51 | - | - | - | - | - |
| *E*-Isocitral (OM) | 1181 | 1177 | 0.63 | - | - | - | - | - | - |
| α-Terpineol (OM) | 1189 | 1186 | - | 2.14 | - | 0.76 | | 0.57 | 0.58 |
| Myrtenol (OM) | 1195 | 1194 | - | 0.69 | 0.92 | - | - | - | - |
| β-Cyclocitral (OM) | 1208 | 1217 | - | - | 2.29 | - | - | - | - |
| Citronelol (OM) | 1227 | 1223 | 1.17 | - | - | - | - | 0.52 | - |
| **Neral (**OM**)** | 1241 | 1235 | **23.84** | - | - | **8.06** | **14.36** | **16.94** | - |
| **Carvone** (OM) | 1243 | 1239 | 0.8 | **30.72** | - | **11.53** | - | **16.62** | **18.47** |
| Geraniol (OM) | 1253 | 1249 | 1.14 | - | - | - | - | - | - |
| **Geranial** (OM) | 1272 | 1264 | **32.31** | - | - | **11.23** | **22.1** | **21.25** | |
| Piperitenone (OM) | 1339 | 1340 | - | 1.34 | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| β-Cubebene (HS) | 1390 | 1387 | - | 0.58 | - | - | - | - | - |
| β-Elemene (HS) | 1391 | 1389 | 0.64 | 0.79 | 2.78 | 0.9 | - | - | - |
| ***E*-Caryophyllene** (HS) | 1419 | 1417 | 0.58 | - | **4.14** | 0.92 | 0.99 | - | 1.02 |
| **γ-Muurolene** (HS) | 1481 | 1478 | **7.67** | **6.45** | **4.13** | **4.82** | **1.98** | **2.48** | **2.16** |
| α-Zingiberene (HS) | 1494 | 1493 | 1.15 | - | - | - | - | - | - |
| Cubebol (OS) | 1514 | 1514 | 0.58 | 1.14 | - | 0.57 | | 0.53 | - |
| **Elemol** (OS) | 1549 | 1548 | **5.39** | **5.21** | - | 3.29 | 1.62 | 2.54 | 0.65 |
| Guaiol (OS) | 1597 | 1600 | - | 0.53 | - | - | - | 0.55 | - |
| Cedr-8(15)-en-9-α-ol (OS) | 1650 | 1650 | - | 1.3 | - | - | - | 0.61 | - |
| 8-Cedren-13-ol (OS) | 1696 | 1688 | - | 1.61 | - | - | - | 0.51 | - |
| Curcumenol (OS) | 1736 | 1733 | - | 0.6 | - | - | - | - | - |
| Hydrocarbon monoterpenes (HM) | | | **19.02** | **18.95** | **9.18** | **30.73** | **18.31** | **20.92** | **27.92** |
| Oxygenated monoterpenes (OM) | | | **45.65** | **34.12** | **55.08** | **30.73** | **45.75** | **36.61** | **41.88** |
| Hydrocarbon sesquiterpenes (HS) | | | **15.21** | **15.16** | **27.54** | **13.36** | **18.31** | **5.23** | **13.96** |
| Oxygenated sesquiterpenes (OS) | | | **3.8** | **22.75** | | **10.24** | **9.15** | **26.15** | **6.04** |
| Esters | | | **19.02** | **18.95** | **9.18** | **30.73** | **18.31** | **20.92** | **27.92** |
| Others | | | | | | | | | |
| Total (%) | | | **91.3** | **91.0** | **91.8** | **87.07** | **91.56** | **88.91** | **90.74** |

Source: Authors (2024).

$RI_{calc}$ = Calculated retention time; $RI_{lit}$ = Retention time claimed in the literature.

*Yield reported by the producer. LA1 = *Lippia alba* citral; LA2 = *Lippia alba* carvone; LA3 = *Lippia alba* linalool; Pool 1 = LA1 + LA2 + LA3; Pool 2 = LA1 + LA3; Pool 3 = LA1 + LA2; Pool 4 = LA2 + LA3.

### 3.2. Qualitative assay of AChE inhibitory activity

Figure 2 shows the halos of inhibition of *L. alba* essential oil samples using the enzyme acetylcholinesterase by direct bioautography assay. All essential oil samples of the different chemotypes (LA1, LA2 and LA3), in addition to the physostigmine standard, showed inhibition halo formation in the chromatoplate.

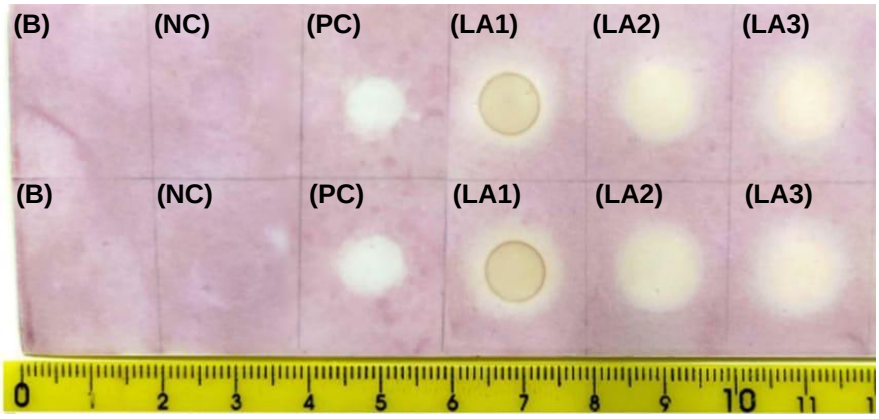


**Figure 2** – Direct bioautography on the silica gel chromatoplate (TLC) of the anticholinesterase activity of *Lippia alba* essential oil (100 µL/mL) and physostigmine samples. LA1 = *Lippia alba* citral; LA2 = *Lippia alba* carvone; LA3 = *Lippia alba* linalool; Controls: (B) blank, (NC) methanol negative control, and (PC) physostigmine positive control (100 µg/mL). Inhibition halos were measured in cm.

### 3.3. Quantitative assay of AChE inhibitory activity

According to the results of Figure 3, it is possible to observe that all samples of *L. alba* essential oil and the different mixtures (pool) showed inhibitory activity against acetylcholinesterase, with an $IC_{50}$ that ranged from 0.1 to 4.3 µg/mL.

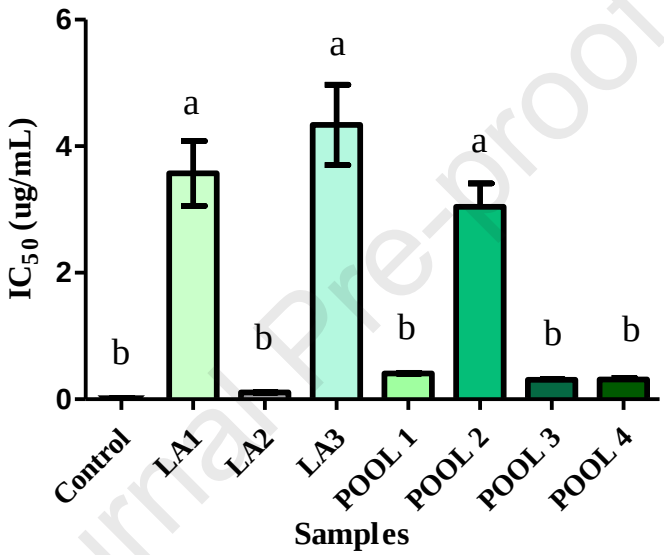



**Figure 3** – Anticholinesterase activity ($IC_{50}$) of *Lippia alba* essential oil samples and their respective mixtures (pools). Control = physostigmine; LA1 = *Lippia alba* citral; LA2 = *Lippia alba* carvone; LA3 = *Lippia alba* linalool; Pool 1 = LA1 + LA2 + LA3; Pool 2 = LA1 + LA3; Pool 3 = LA1 + LA2; Pool 4 = LA2 + LA3.

### 3.4. Redocking of donepezil into the active site of AChE (PDB ID: 4EY7)

The human AChE enzyme co-crystallized with donepezil (PDB ID: 4EY7) was used as a virtual target to classify the main constituents of the essential oil samples according to their binding affinities. First, the anchoring procedures were validated by precisely refitting the co-crystallized donepezil into the *h*AChE model to better compare our anchoring results. Donepezil was anchored against AChE with the same parameters and was observed to have -11.237 kcal/mol as the binding affinity (Figure 4 and Table 4). The energy calculation for all anchor complexes was evaluated using the MMFF94 force field as the scoring function.

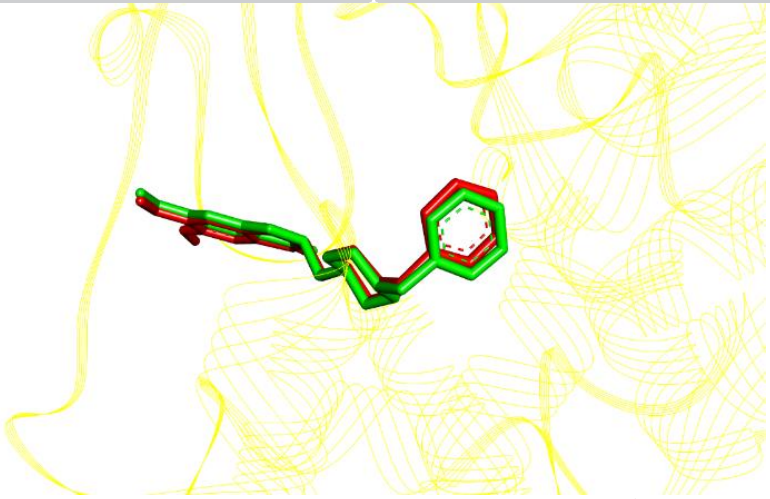


**Figure 4** – Re-docking of donepezil and *h*AChE (4EY7 and donepezil). In green, experimental pose and, in red, predicted *in silico* pose. **RMSD:** 0.334**;** ΔG= -11.237 kcal/mol.

Figure 5 shows the binding pocket and target residues involved in the binding interaction of donepezil.

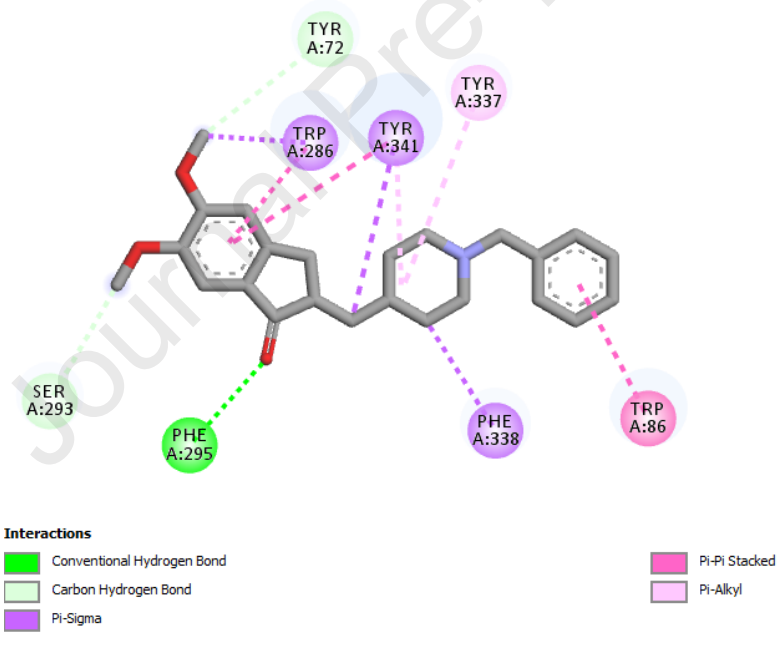



**Figure 5** – Donepezil and AChE.

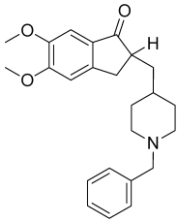
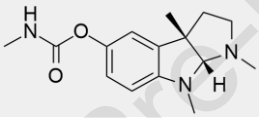
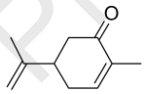
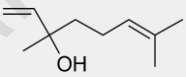
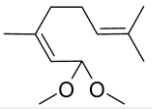
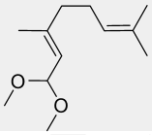
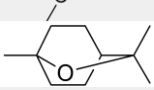
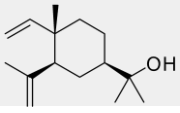
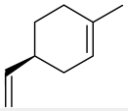
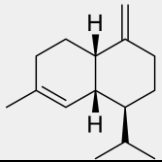
## 3.5 Molecular docking validation

In addition to re-docking of donepezil to *h*AChe, we also used physostigmine as a positive control for molecular docking studies. We used the same donepezil coordinates, as there is no co-crystal with human acetylcholinesterase deposited in the database. The best pose showed binding affinity of -9,146 Kcal/mol as can be seen in Table 4.

382 3.6. Binding affinity and binding pocket analysis of the phytochemicals

383 The energy calculation for all anchor complexes was evaluated using the MMFF94
384 force field as the scoring function. This study evaluated the results of ten poses obtained
385 through molecular docking on the DockThor Portal, and we considered the ranked pose with
386 the highest binding affinity value. The results can be viewed in Table 4.

387 **Table 4** – Screening of the most abundant constituents of *Lippia alba*.

| Ligand | Chemical structure | AChE binding affinity (kcal/mol) |
|---|---|---|
| Donepezil (Pivot) |  | -11.237 |
| Physostigmine |  | -9,146 |
| Carvone |  | -7.951 |
| Linalool |  | -8,114 |
| Neral dimethyl acetal |  | -8.218 |
| Geranial dimethyl acetal |  | -8.476 |
| 1,8-Cineole |  | -7.960 |
| Elemol |  | -9.252 |
| Limonene |  | -8.194 |
| γ-Muurolene |  | -9.134 |

388

In relation to the chemotypes present in *L. alba*, the binding affinity values ranged from -8,095 to -9,217 Kcal/mol exhibited by the coupling of limonene and elemol, respectively. Figure 6 correlates the average binding affinity values of the phytochemicals with the reference ligand donepezil. It is noted that there was a statistically significant difference between all molecules and donepezil. However, as can be seen in the 2D diagrams (Figures 7-12), the compounds established important interactions with some of the amino acid residues present in the active site of AChE enzyme and which participate in the complexation with donepezil.

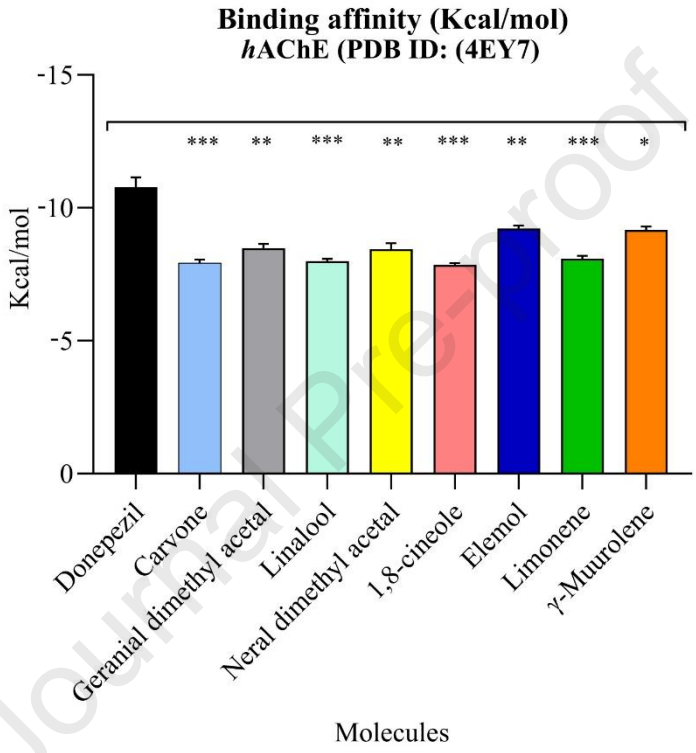



**Figure 6** – Statistical analysis of the binding energy values from donepezil compared to those of the phytochemicals.

## 3.7. Binding pocket analysis of the phytochemicals

The docking complexes of the main constituents were examined in order to interpret the binding conformation pattern within the active site of AChE (4EY7) compared to donepezil. Figure 7 shows the binding pocket and target residues involved in the binding interaction of donepezil.

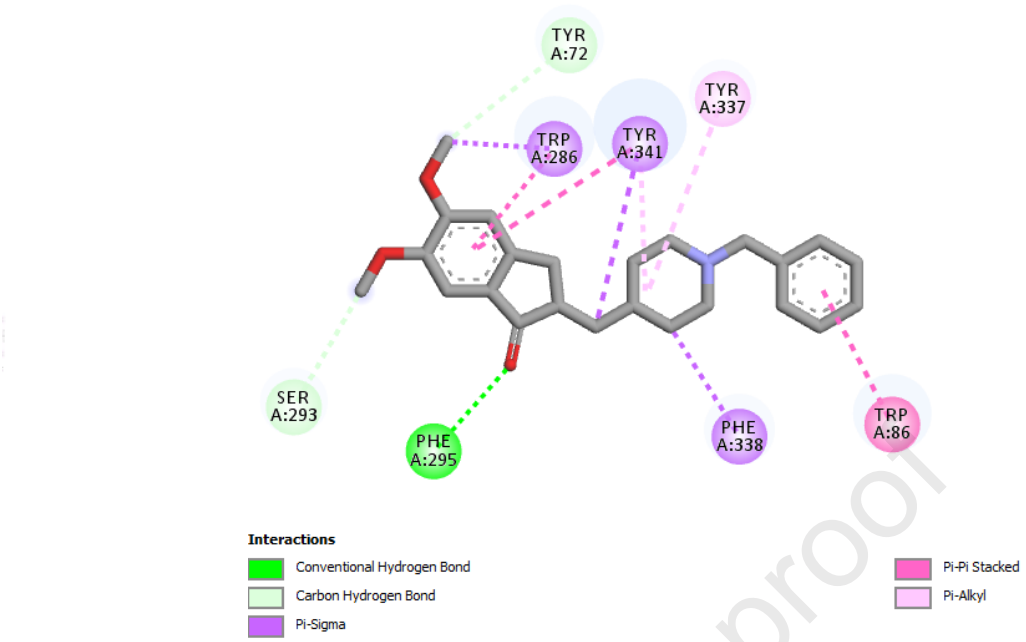



**Figure 7** – Donepezil and AChE.

Figure 8 presents the involved amino acids residues of *h*Ache with physostigmine. Previous studies showed that its accommodation to the active site is mediated especially by hydrophobic interactions [49].

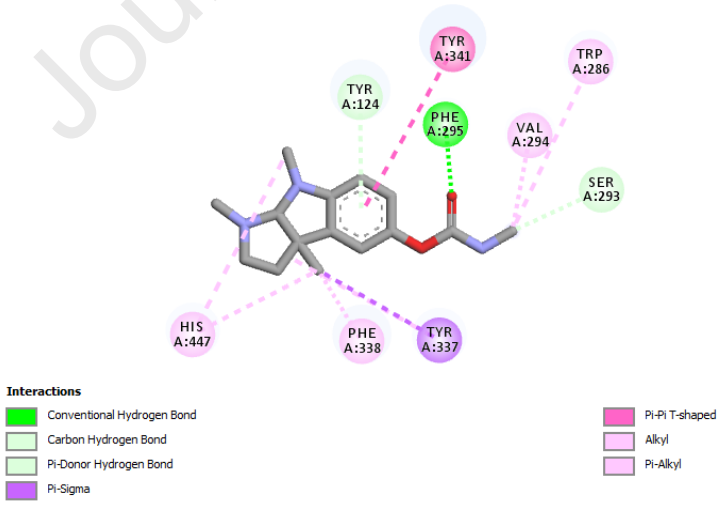



**Figure 8** – Physostigmine and AChE.

Figure 9 shows the interaction diagram with amino acid residues of the monoterpenes carvone (A) and elemol (B).

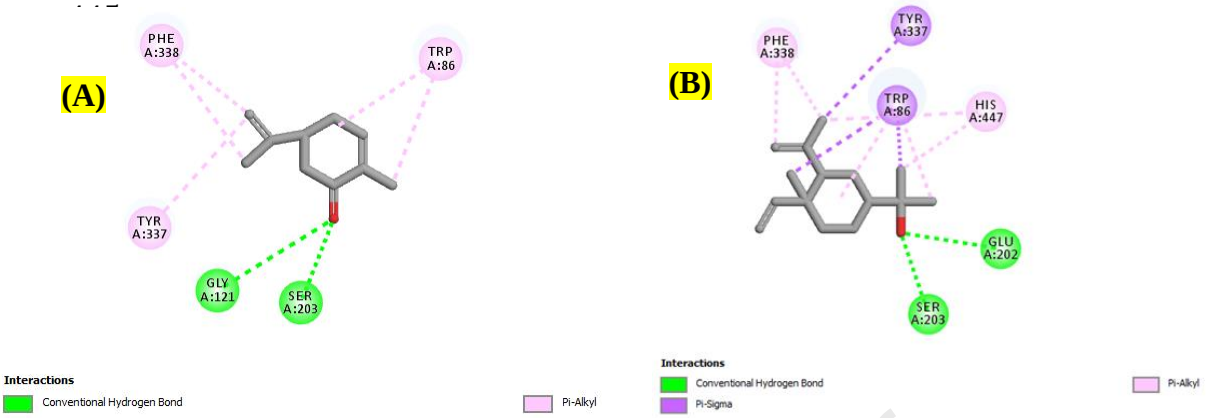



**Figure 9** – 2D Diagram of the interactions between carvone (A) and elemol (B) and amino acid residues from *h*AChE (4EY7).

Figure 10 shows the bonds made with the amino acid residues of AChE by the terpene hydrocarbon limonene (A), and sesquiterpene γ-muurolene (B).

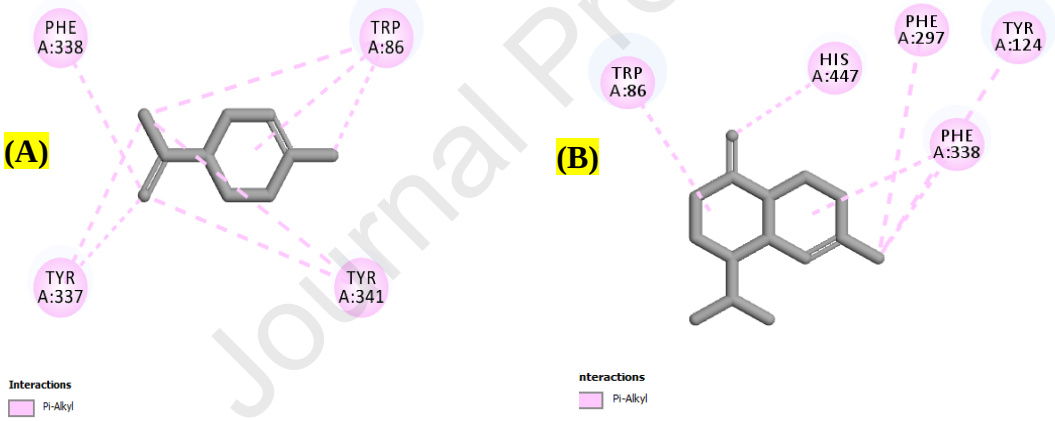



**Figure 10** – 2D diagram of the interactions between limonene (A) and γ-muurolene (B) and amino acid residues from *h*AChE (4EY7).

Figure 11 shows the bonds made with the amino acid residues of AChE by the oxygenated monoterpenes neral dimethyl acetal (A) and geranial dimethyl acetal (B).

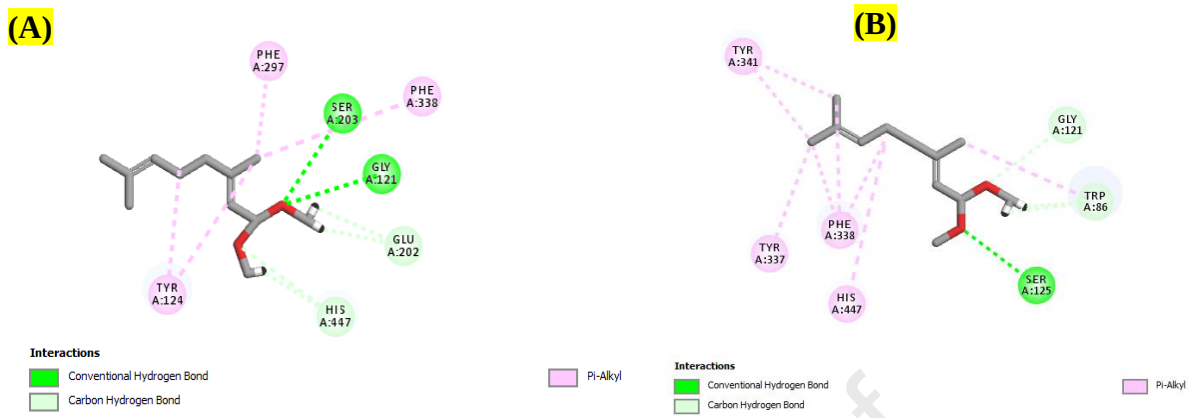



**Figure 11** – 2D diagram of the interactions between neral dimethyl acetal (A) geranial dimethyl acetal (B) and amino acid residues from *h*AChE (4EY7).

Figure 12 shows the bonds made with the amino acid residues of AChE by the cyclic compound 1,8-cineole (A) and by the monoterpene linalool (B).

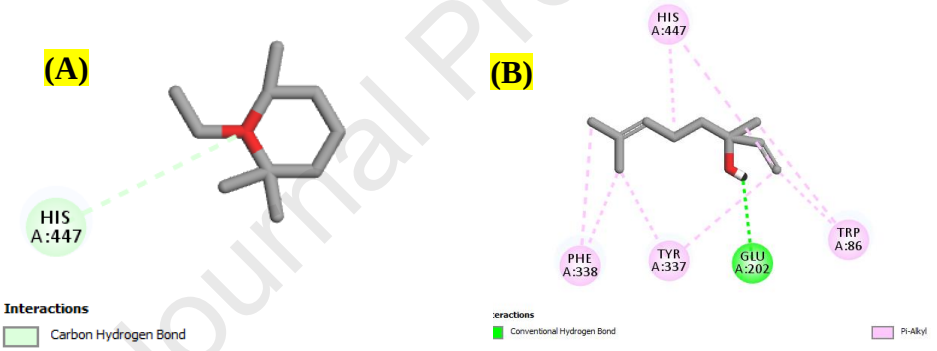



**Figure 12** – 2D diagram of the interactions between 1,8-cineole (A) linalool (B) and amino acid residues from *h*AChE (4EY7).

## 3.6. Pharmacokinetic prediction

The screened phytochemicals were subjected to pharmacokinetic analysis using the online prediction tool Swiss ADME. The results are presented in Table 5.

**Table 5** – *In silico* prediction of pharmacokinetic properties of the natural compounds from *Lippia alba*.

| Ligand | MW (g/mol) | NRB | HBA | HBD | Log $P_{o/w}$ | GI absorption | BBB permeation |
|---|---|---|---|---|---|---|---|
| Donepezil | 379.49 | 6 | 4 | 0 | 4.00 | High | Yes |
| Carvone | 150.22 | 1 | 1 | 0 | 2.44 | High | Yes |
| Linalool | 154.25 | 4 | 1 | 1 | 2.66 | High | Yes |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Neral dimethyl acetal | 198.30 | 6 | 2 | 0 | 3.05 | High | Yes |
| Geranial dimethyl acetal | 198.30 | 6 | 2 | 0 | 3.07 | High | Yes |
| 1,8-Cineole | 154.25 | 0 | 1 | 0 | 2.67 | High | Yes |
| Elemol | 222.37 | 3 | 1 | 1 | 3.77 | High | Yes |
| Limonene | 136.23 | 1 | 0 | 0 | 3.35 | Low | Yes |
| γ-Muurolene | 204.35 | 1 | 0 | 0 | 4.18 | Low | No |

MW = molecular weight; NRB = number of rotatable bonds; HBA = number of hydrogen bond acceptors; HBD = hydrogen bond donors; Log $P_{o/w}$ = octanol/water partition coefficient; GI = relative gastrointestinal absorption and BBB = blood brain barrier.

## 4. Discussion

The objective of this study was to evaluate whether the essential oil from the three chemotypes of *Lippia alba* and mixtures of these has an inhibitory effect on the enzyme acetylcholinesterase (AchE), as well as to determine via the molecular anchoring technique the best energies of receptor-ligand interaction of the main constituents present in the oil samples with AChE. In this regard, it has been shown that, according to the main constituents (citral chemotype - LA1; chemotype carvone - LA2 and chemotype linalool - LA3), the chemotypes significantly ($p \leq 0.05$) inhibited the action of AChE, with the chemotype rich in carvone (LA2) with an $IC_{50}$ of 0.10 µg/mL ± 0.006 being the most active in relation to the others (LA1 $IC_{50}$ 3.57 µg/mL ± 0.51 and LA3 $IC_{50}$ 4.34 µg/mL ± 0.63). These results of anticholinesterase action can be seen in figures 2 and 3. In addition, it was observed that the mixtures containing LA2, these being pool 1 ($IC_{50}$ 0.41 µg/mL ± 0.008); pool 3 ($IC_{50}$ 0.31 µg/mL ± 0.023) and pool 4 ($IC_{50}$ 0.31 µg/mL ± 0.035) stood out in relation to the mixture containing only the chemotypes LA1 and LA3 (Figure 3). Although all chemotypes showed significant inhibitory action ($p \leq 0.05$) of AChE, the presence of carvone seems to potentiate this action. The molecular docking study complemented the results of the experiment and demonstrated that there are significant interactions between the main constituents of the oils and the amino acid residues of the AChE enzyme.

The molecular anchoring experiment is a technique that consists of predicting the orientation and binding conformation of ligands in the active region of target proteins. In this study, the human AChE enzyme, co-crystallized with donepezil (PDB ID: 4EY7), was used as a virtual target to classify the main constituents of the essential oil samples according to their

binding affinities. This crystal was chosen since donepezil is commonly known as a cholinesterase inhibitor and is used in the treatment of Alzheimer's disease as a standard drug. First, the anchoring procedures were validated by precisely refitting the co-crystallized donepezil into the *h*AChE model to better compare our anchoring results. Donepezil was anchored against AChE with the same parameters and was observed to have -11.237 kcal/mol as the binding affinity (Figure 4). The energy calculation for all anchoring complexes was evaluated using the MMFF94 force field as the scoring function. Based on the results of the energy values, eight main constituents of the oil samples (carvone, linalool, neral dimethyl acetal, geranial dimethyl acetal, 1,8-cineole, elemol, limonene, γ-murolene) showed energy values that were close to that of donepezil, though they were significantly different ($p \leq 0.05$) (Table 4 and Figure 6).

Physostigmine is a drug widely used in the treatment of Alzheimer's disease. In this study, it was found that the interactions with the catalytic site are mostly hydrophobic, with only one hydrogen bond between the carbamate group and Phe295. π-alkyl and alkyl interactions are observed with residues Trp286, Val294, Phe338 and His447. Other interactions, such as π-π-shaped and π-sigma, are shown with residues Tyr341 and Tyr337, respectively. The compounds γ-muurolene and elemol presented binding affinity values very close to those calculated for physostigmine.

The docking complexes of the main constituents were examined in order to interpret the binding conformation pattern within the active site of AChE (4EY7) when compared to donepezil. Figure 7 shows the binding pocket and target residues involved in the binding interaction of donepezil. As noted, ten amino acid residues are the counterpart of the binding site: Trp286, Tyr72, Tyr124, Tyr341, Phe338, Tyr337, Glu202, Ser203, Trp86 and His447. Natural compounds that have the potential to bind to these residues can be considered AChE inhibitors.

As seen in Figure 9A, carvone, a main constituent of LA2, interacts with Gly121 through hydrogen bonding, unlike donepezil. This result also differs from that established by Wojtunik-Kulesza et al. (2017) [50], who via molecular docking evaluated the interactions of different monoterpenes, such as carvone, that are present in vegetable oils and showed that the carbonyl of hydrogen-bonded carvone interacts with Tyr337 [51]. Various reasons can be given to explain these divergences, which include the choice of crystallographic structure and the molecular docking protocol. On the other hand, in our study, the π-alkyl and π-sigma interactions of carvone with trp86, Tyr337, His447 and Phe338 residues are the same as those of donepezil (Figures 9A and 8).

The hydrocarbons terpenes, limonene (figure 10A), present in LA1 and LA2, and γ-muurolene (figure 10B), present in LA1, LA2 and LA3, are apolar compounds, so they bind to AChE only through π-alkyl hydrophobic interactions. Monoterpene limonene interacts with Phe338, Trp86, Tyr341 and Tyr37 while the sesquiterpene γ-muurolene interacts with five residues: Phe338, Trp86, His447, Phe297, Tyr124 (Figure 10B). As seen in Figure 11, the oxygenated monoterpenes, neral (figure 11A) and geranial (figure 11B), which are main constituents of LA1, interact by bonding of hydrogen, π-alkyl and hydrogen-carbon bonding. The monoterpene neral interacts through hydrogen bonding with Ser203 and Gly121, while geranial interacts with the residue of Ser125. The π-alkyl interactions for neral occur via the residues Phe297, Phe338 and Tyr124, while for the compound geranial, the interactions occur via the residues Tyr341, Phe338, His447 and Tyr337.

Cyclic constituents, such as 1,8-cineole (figure 12A) found in essential oils, have been reported to be AChE inhibitors. The synergistic associations of these sesquiterpenes may be responsible for their inhibitory action [52]. Linalool (figure 12B) interacts with a hydrogen bond of the AChE in Glu202 and via π-alkyl hydrophobic interactions with the residues Trp86, Hit447, Tyr337, Phe338, similarly to donepezil (Figure 12). These interactions are in agreement with the results of the biological evaluations and confirm the significant activity of the essential oil of the different *L. alba* chemotypes when tested against AChE.

Previous studies have reported that the inhibitory activity against the enzyme acetylcholinesterase is related to the high content of monoterpenes in the chemical composition, such as 1,8-cineole, α-pinene, linalool and caryophyllene oxide [53,54]. In our study, 1,8-cineole and α-pinene are present in the chemotype LA2 and linalool and caryophyllene oxide are present in chemotype LA3. Although α-pinene and caryophyllene oxide do not exceed 2% of the constituents found, 1,8-cineole is one of the main constituents of chemotype LA2 and the mixtures (pools 1, 3 and 4). Linalool is present as a main component in the LA3 chemotype. One study that evaluated the anticholinesterase activity of the essential oil of a plant native to Malaysia (*Pseuduvaria macrophylla*), which does not have monoterpenes in its composition, showed a weak inhibition against acetylcholinesterase and butyrylcholinesterase [55]. In other words, the volatile constituents of essential oils can have different biological properties and the synergism between the constituents is important for potentiating the anticholinesterase effect.

In addition, other studies have reported that the essential oil of citrus species that present limonene, citronellol, o-cymene and 1,8-cineole in their chemical composition tend to exhibit strong inhibition of acetylcholinesterase [56,57], which corroborates the findings of

our research. One study that evaluated the anticholinesterase action of monoterpenes highlighted S-carvone and linalool among the main inhibitors found, as well as fenchone, γ-terpinene, geraniol, estragol and camphor [58].

Studies evaluating the anticholinesterase activity of *Lippia alba* essential oil [23,24] and its extracts [25] have shown the species to have a potential cholinesterase inhibitory effect. The variety of effects attributed to *Lippia alba* is a result that is related in part to its volatile constituents. Morais et al. (2022) [59], who evaluated the anticholinesterase activity of *L. Alba* chemotypes, presented different results in relation to the present study, since the chemotype with the lowest $IC_{50}$ was citral, unlike in our research, in which the carvone chemotype was the most active. However, it is important to note that the other constituents present in the essential oil differ from that presented in this study. Since essential oils are mixtures of several compounds that can act synergistically and antagonistically with each other, the efficacy of the samples as acetylcholinesterase inhibitors may also vary depending on the percentage of constituents that are in smaller quantities, which could explain the difference between these two studies. In addition, it is important to highlight that many factors can influence the variation of the chemical constitution and, consequently, the biological activity of a plant species. Thus, it is almost impossible to obtain the same results, or even similar ones, despite using the same chemotype of a plant.

Pharmacokinetic analyses of natural compounds are important steps in the classification of molecules based on absorption, distribution, metabolism, and excretion (ADME). The main constituents of the chemotypes LA1, LA2 and LA3 showed properties similar to oral drugs and follow Lipinski's rule of five. The logP value of all the constituents is less than five, the hydrogen bond donor and acceptor atoms are in the optimal range, and the molecular weights are less than 500 Da. These results corroborate those found by Awasthi et al. (2017) [53], who predicted the pharmacokinetic characteristics of 25 promising terpenoids, including carvone, limonene, linalool and geraniol, against Alzheimer's disease. In addition, drugs that act on the central nervous system have significantly reduced molecular weights compared to other therapies. Previous work has reported a cutoff for blood-brain barrier (BBB) penetration of up to 400 Da. Penetration into the CNS requires a sum of heteroatoms of five or less and has significantly fewer rotatable bonds than other classes of drugs, usually the number of rotatable bonds is five or less. These drugs have less than three H-binding donors and the number of H-binding acceptors is less than seven. All of the compounds mentioned above fit these criteria, so they are able to permeate the BBB and reach the target. However, limonene and γ-muurolene do not meet the water solubility criteria, so

these compounds probably have low oral bioavailability and are not suitable as potential oral drug candidates. Thus, six of the main compounds of *Lippia alba* have great physicochemical properties that can be developed as a potential oral drug for Alzheimer's disease.

## 5. Conclusion

The essential oil of *Lippia alba*, which has chemotypes that are rich in citral, carvone, and linalool, has inhibitory action against the enzyme acetylcholinesterase, with the chemotype that is rich in carvone being the most active. The molecular docking study complemented the experimental results and demonstrated significant interactions between the main constituents of essential oils and the amino acid residues of the AChE enzyme. The pharmacokinetic studies revealed that six of the main constituents from *L. alba* have properties that are similar to oral drugs and follow Lipinski's rule of five. In addition, their reduced molecular weights allow these compounds to achieve the central nervous system. Thus, six of the main compounds of *Lippia alba* have great physicochemical properties that can be developed as a potential oral drug for Alzheimer's disease.

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## Author contributions

RHVM was responsible for the development of the concept and hypotheses of this research, writing of the manuscript, raw material used for the samples and data analysis. AQSJ conducted the analyses of the volatile constituents of the essential oil samples and conducted the experiments of the anticholinesterase tests, data analysis and drafted the manuscript. KAS conducted the biological experiments and contributed to the writing of the manuscript. LEMB was responsible for the development of the concept and hypotheses for this research,

performed the data analysis and edited the manuscript. ASB was responsible for determining the chemical composition of the oil samples. GBS was responsible for the molecular modeling and contributed to the writing of the manuscript. GSR was responsible for molecular modeling and determination of the pharmacokinetic parameters of chemical compounds. All authors read and approved the final version of the manuscript.

**Declaration of interests**

The authors declare that there are no conflicts of interest.

**Data availability statement**

The data associated with this study has not been deposited in a publicly available repository; however, it can be provided upon request.

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: