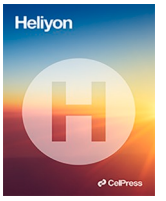
Heliyon 10 (2024) e29063

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

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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ARTICLE INFO

Keywords:
Erva cidreira
Aromatic plant
Acetylcholinesterase inhibitors

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.

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

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https://doi.org/10.1016/j.heliyon.2024.e29063
Received 14 August 2023; Received in revised form 27 March 2024; Accepted 28 March 2024
Available online 3 April 2024

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 (Fig. 1). However, 1,8-cineole, myrcene and β-elemene also occur [16–18]. 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].

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 [37].

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.

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

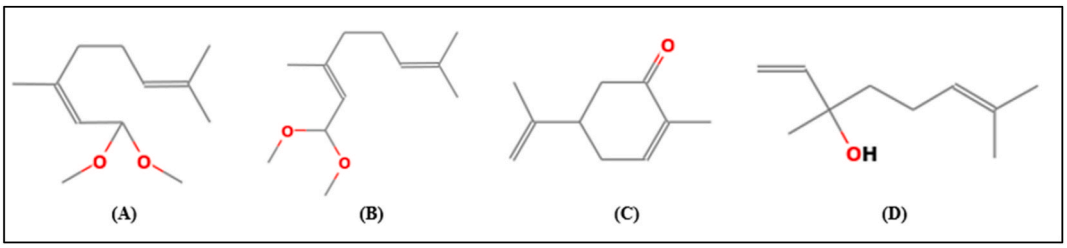


Fig. 1. Chemical structure of the majority components of the main chemotypes of *L. alba.* (A) = citral - neral dimethyl acetal; (B) citral - geranial dimethyl acetal; (C) = carvone and (D) = linalool. Source: NIST.

Table 1
Main studies that evaluated the biological activities of the essential oil from different chemotypes of *L. alba*, published in the period 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	[23]
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	[24]
3	Quintero et al.	Immunomodulatory, trypanocide, and antioxidant properties of essential oil fractions of *Lippia alba* (Verbenaceae).	Citral and carvone	Trypanocide and immunomodulator	2021	[25]
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	[26]
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	[27]
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	[28]
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	[29]
8	Gomes et al.	*Lippia alba* and *Lippia gracilis* essential oils affect the viability and oviposition of *Schistosoma mansoni*.	Citral	Anthelminth	2022	[30]
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	[31]
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	[32]
11	Pagotti et al.	Trypanocidal Activity of *Dysphania ambrosioides*, *Lippia alba*, and *Tetradenia riparia* Essential Oils against *Trypanosoma cruzi*.	Linalool	Trypanocide	2021	[33]
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	[34]
13	de Brito et al.	Identification of Bioactive Compounds against *Aedes aegypti* (Diptera: Culicidae) by Bioassays and in Silico Assays.	Citral	Repellent	2021	[35]
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	[36]

Source: Authors (2024).

and LA2) were collected in the village of Alter do Chão (2°30′31.0″ S and 54°57′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 h.

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 °C 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 [27] and a fused silica capillary column (Rxi-5ms, Restek Corporation, Bellefonte, PA,

USA) of 30 m × 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 °C; oven temperature program: 60–240 °C; ion source temperature: 200 °C; transfer line temperature: 250 °C.

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

2.4.2. Quantitative assay

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

In a total volume of 1 mL, 415 μL of buffer A, 10 μL of the essential oil solution (diluted in methanol, buffer and Tween 80) at different concentrations (100, 50, 25, 12.5, 6.25 and 3.12 μg/mL), and 75 μL of acetylcholinesterase enzyme, containing 0.2 U/mL, were added. The samples were then incubated for 15 min at 25 °C. After incubation, 75 μL of a solution of 1.83 mM AChI (acetylthiocholine iodide) (Sigma-Aldrich, Steinheim, Germany) and 425 μL of 3 mM DTNB (5,5′-dithiobis [2-nitrobenzoic acid]) (Sigma-Aldrich, Steinheim, Germany) were added and the mixture was incubated for 30 min at 25 °C under a light source. The absorbance of the mixture was measured at 412 nm in a UV spectrophotometer (NOVA, 3300). Physostigmine was used as the standard drug and a dilution solution was used as negative control (buffer A, methanol and Tween 80 at a ratio of 2:2:1). The percentage of inhibition of enzyme activity was calculated according to the equation % = [(A0 – A1)/A0] * 100, where A0 was the absorbance of the control without the essential oil and A1 was the absorbance of the essential oil sample at different concentrations. All tests were performed in triplicate. The concentration of the sample that provided 50% inhibition (IC_{50}) was obtained by constructing graphs of the percentages

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

LA1 = *Lippia alba* citral chemotype; LA2 = *Lippia alba* carvona chemotype; LA3 = *Lippia alba* linalool chemotype.
Source: Authors (2024).

of inhibition versus the concentration of the inhibitor. The non-linear regression parameters for the curve were plotted and the IC_{50} values were obtained using the Microsoft Excel 2019 software.

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

To evaluate the possible synergistic effect of the different essential oil samples of the chemotypes of *L. alba* on the acetylcholinesterase enzyme, mixtures of the three oil samples 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.

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 20 × 20 × 20 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

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

Essential oils Yield			LA1	LA2	LA3	Pool 1	Pool 2	Pool 3	Pool 4
			3.7%	1.1%	1.3%[a]	–	–	–	–
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**

RI_{calc} = Calculated retention time; RI_{lit} = Retention time claimed in the literature.
[a] 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.
Source: Authors (2024).

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

3.2. Qualitative assay of AChE inhibitory activity

Fig. 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.

3.3. Quantitative assay of AChE inhibitory activity

According to the results of Fig. 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.

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 (Fig. 4 and Table 4). The energy calculation for all anchor complexes was evaluated using the MMFF94 force field as the scoring function.

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

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 −9146 kcal/mol as can be seen in Table 4.

3.6. Binding affinity and binding pocket analysis of the phytochemicals

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

In relation to the chemotypes present in *L. alba*, the binding affinity values ranged from −8095 to −9217 kcal/mol exhibited by the

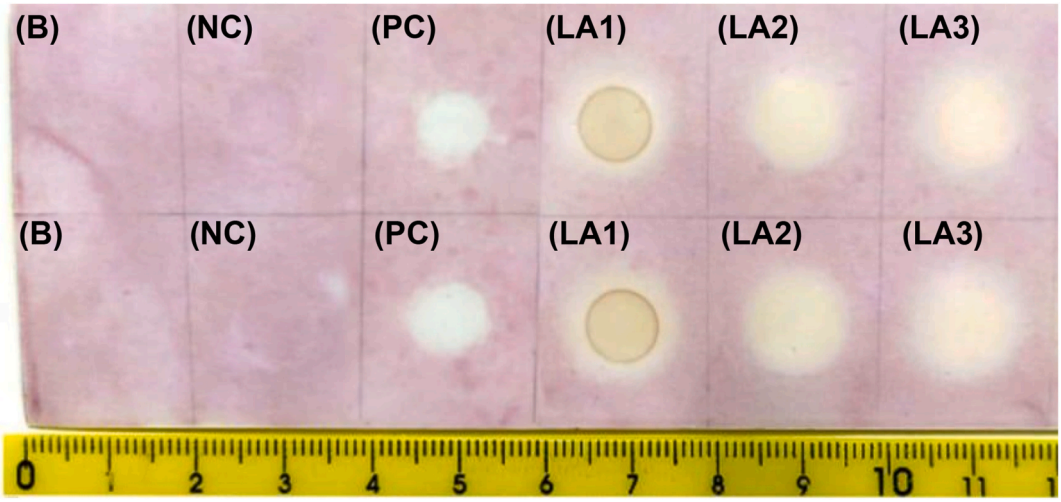


Fig. 2. Direct bioautography on the silica gel chromatoplate (TLC) of the anticholinesterase activity of *Lippia alba* essential oil (100 μL/mL) and physostigmine samples. LA1 = *Lippia alba* citral; LA2 = *Lippia alba* carvone; LA3 = *Lippia alba* linalool; Controls: (B) blank, (NC) methanol negative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

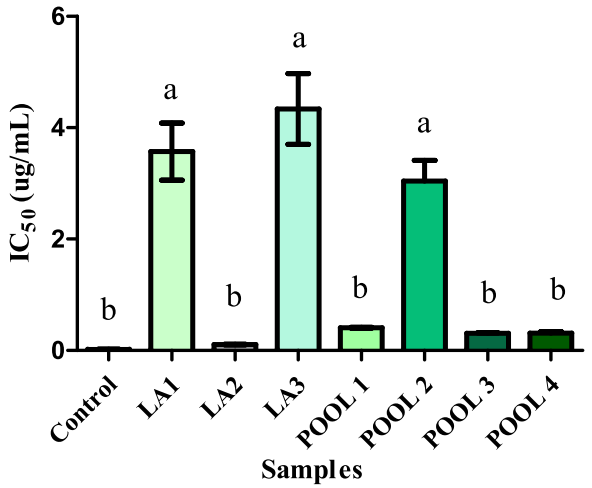


Fig. 3. Anticholinesterase activity (IC_{50}) of *Lippia alba* essential oil samples and their respective mixtures (pools). Control = physostigmine; LA1 = *Lippia alba* citral; LA2 = *Lippia alba* carvone; LA3 = *Lippia alba* linalool; Pool 1 = LA1 + LA2 + LA3; Pool 2 = LA1 + LA3; Pool 3 = LA1 + LA2;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

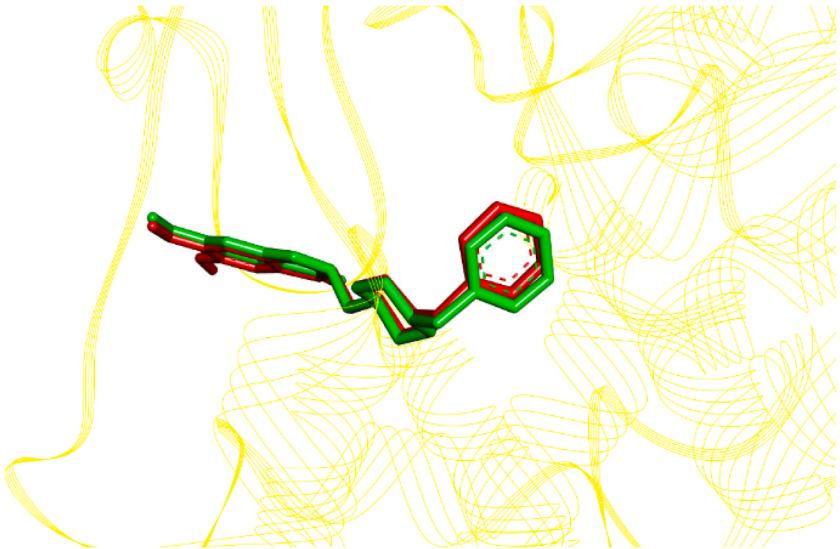


Fig. 4. Re-docking of donepezil and *h*AChE (4EY7 and donepezil). In green, experimental pose and, in red, predicted *in silico* pose. **RMSD:** 0.334; ΔG = −11.237 kcal/mol.

coupling of limonene and elemol, respectively. Fig. 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 (Figs. 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.

3.7. Binding pocket analysis of the phytochemicals

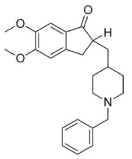
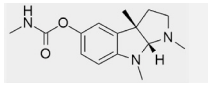
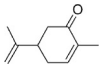
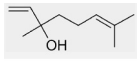
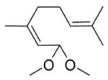
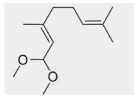
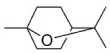
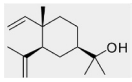
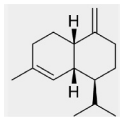
The docking complexes of the main constituents were examined in order to interpret the binding conformation pattern within the active site of AChE (4EY7) compared to donepezil. Fig. 7 shows the binding pocket and target residues involved in the binding interaction of donepezil.

Fig. 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].

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

Fig. 10 shows the bonds made with the amino acid residues of AChE by the terpene hydrocarbon limonene (A), and sesquiterpene

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

Ligand	Chemical structure	AChE binding affinity (kcal/mol)
Donepezil (Pivot)		−11.237
Physostigmine		−9146
Carvone		−7.951
Linalool		−8114
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

γ-muurolene (B).

Fig. 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).

Fig. 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).

3.8. Pharmacokinetic prediction

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

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

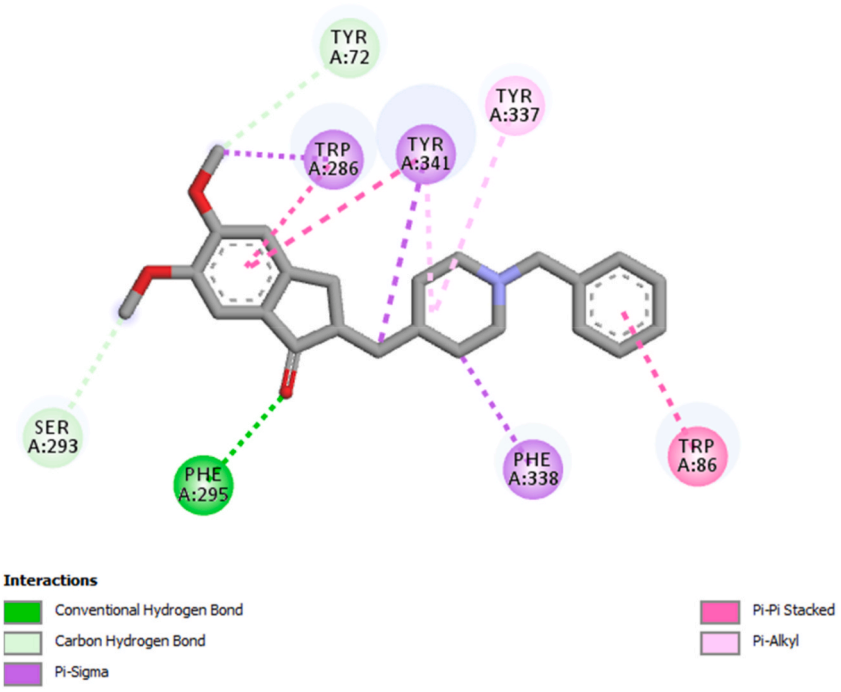


Fig. 5. Donepezil and AChE.

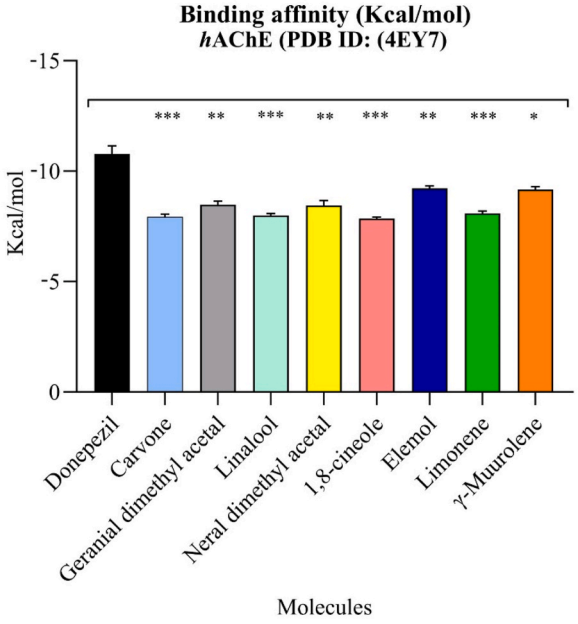


Fig. 6. Statistical analysis of the binding energy values from donepezil compared to those of the phytochemicals.

$\pm$ 0.006 being the most active in relation to the others (LA1 IC_{50} 3.57 μg/mL $\pm$ 0.51 and LA3 IC_{50} 4.34 μg/mL $\pm$ 0.63). These results of anticholinesterase action can be seen in Figs. 2 and 3. In addition, it was observed that the mixtures containing LA2, these being pool 1 (IC_{50} 0.41 μg/mL $\pm$ 0.008); pool 3 (IC_{50} 0.31 μg/mL $\pm$ 0.023) and pool 4 (IC_{50} 0.31 μg/mL $\pm$ 0.035) stood out in relation to the mixture containing only the chemotypes LA1 and LA3 (Fig. 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

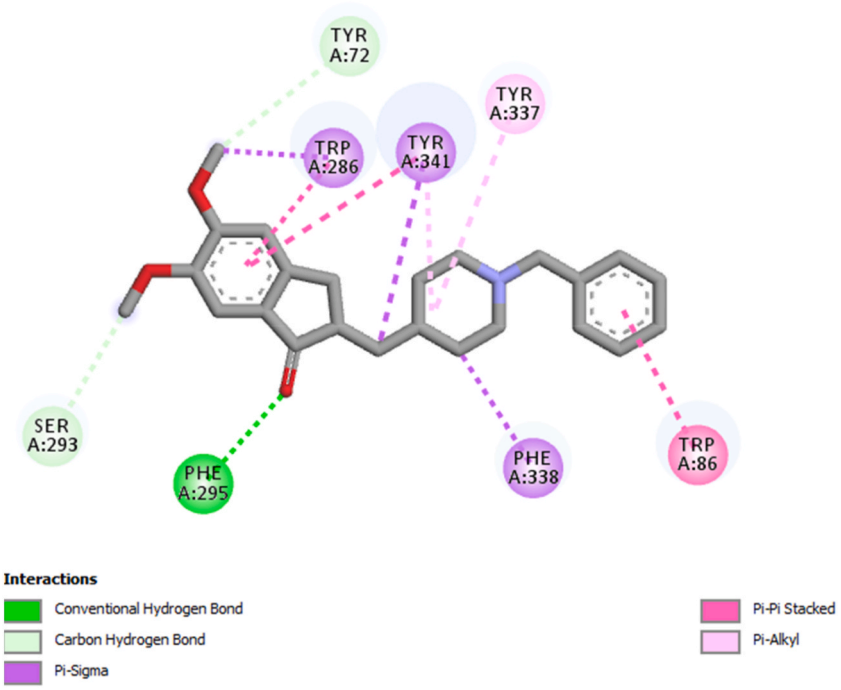


Fig. 7. Donepezil and AChE.

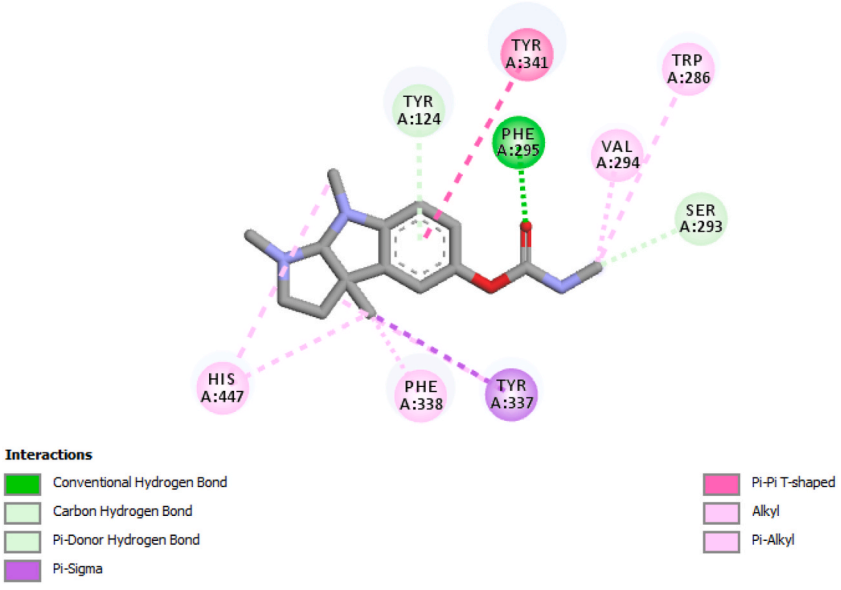


Fig. 8. Physostigmine and AChE.

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 (Fig. 4). The energy calculation for all anchoring complexes was evaluated using the MMFF94

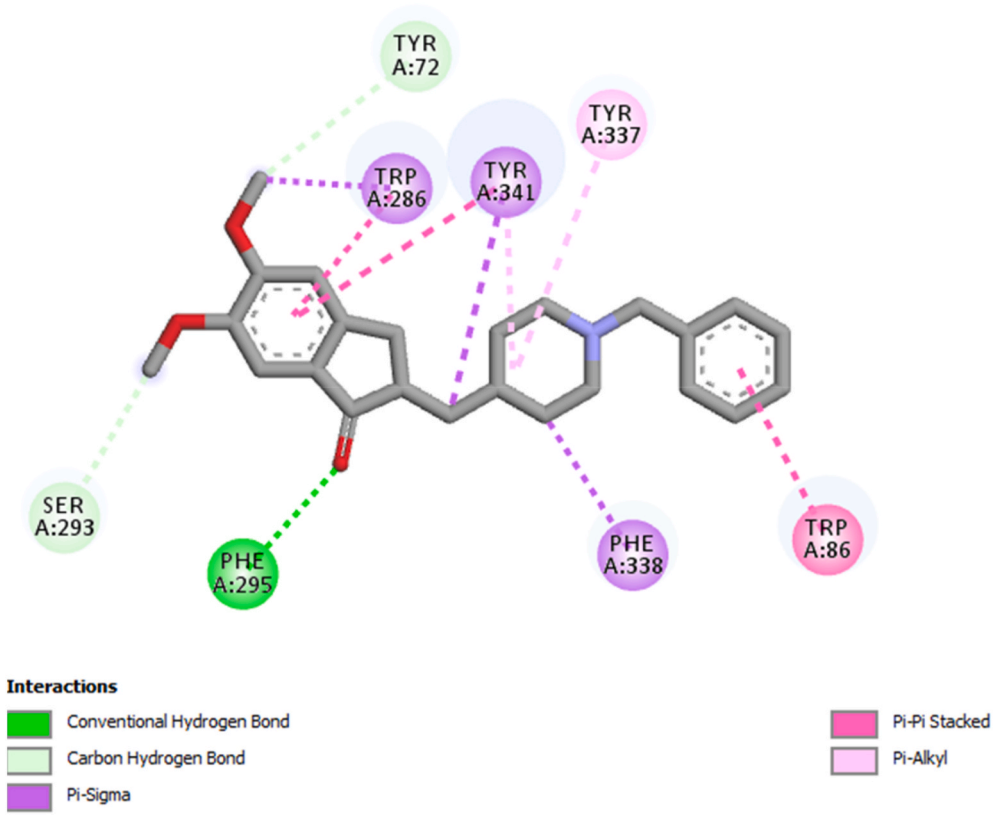


Fig. 9. 2D Diagram of the interactions between carvone (A) and elemol (B) and amino acid residues from *h*AChE (4EY7).

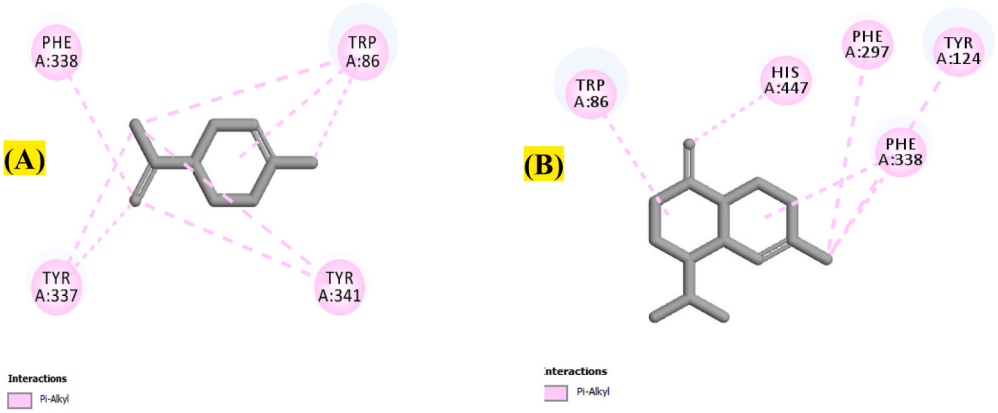


Fig. 10. 2D diagram of the interactions between limonene (A) and γ-muurolene (B) and amino acid residues from *h*AChE (4EY7).

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 Fig. 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. Fig. 7 shows the binding pocket and target residues involved in the binding

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

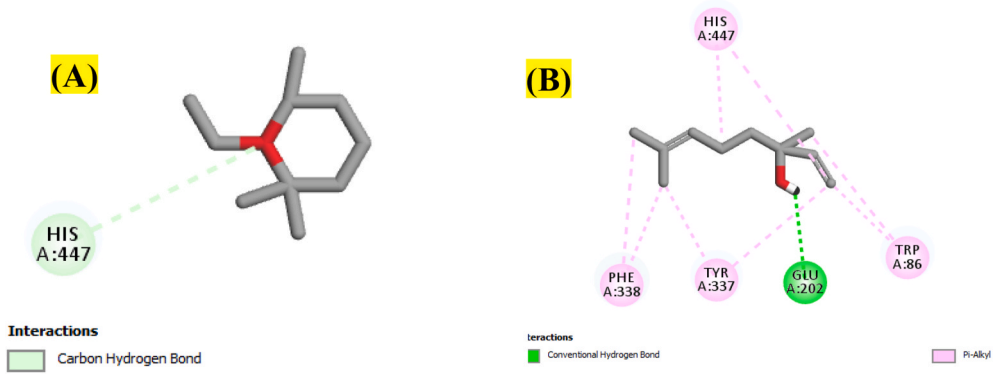


Fig. 12. 2D diagram of the interactions between 1,8-cineole (A) linalool (B) and amino acid residues from *h*AChE (4EY7).

Tables 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.

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 Fig. 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 (Fig. 9A and 8).

The hydrocarbons terpenes, limonene (Fig. 10A), present in LA1 and LA2, and γ-muurolene (Fig. 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 (Fig. 10B). As seen in Fig. 11, the oxygenated monoterpenes, neral (Fig. 11A) and geranial (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 [37,23] and its extracts [24] 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.

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.

CRediT authorship contribution statement

Antônio Quaresma Silva Júnior: Writing – original draft, Methodology, Investigation, Data curation. **Gabriela dos Santos Rodrigues:** Methodology, Investigation, Data curation. **Karina Alcântara de Sousa:** Methodology, Investigation, Data curation. **Leoneide Erica Maduro Bouillet:** Resources, Methodology, Investigation. **Gabriela Bianchi dos Santos:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Adenilson de Sousa Barroso:** Methodology, Investigation. **Rosa Helena Veras Mourão:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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.

Acknowledgments

This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) via the project "Consolidação da Pós-Graduação em Ciências da Saúde com foco em Biotecnologia e Inovação Terapêutica para a melhoria da saúde" (Consolidação da Pós-Graduação em Ciências da Saúde com foco em Biotecnologia e Inovação terapêutica para atenção à saúde), Programa PDPG – Amazônia Legal (Processo 88887.510169/2020-00 – SCBA); CNPq (Processo 309937/2019-1) and the Fundação Amazônia Paraense de Amparo à Pesquisa (FAPESPA/FIAM) and the Programa de Apoio à Redação de Teses, 2020 (Programa de Fomento à Elaboração de Teses 2021 (PRO-Tese, REDE BIONORTE, UFOPA/ISCO).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29063.

References

[1] F.C. Camillo, *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson uma espécie nativa promissora para a introdução em programas nacionais de plantas medicinais e fitoterápicos, Rio de Janeiro, Supl, Revista Fitos (2016) 1–62, https://doi.org/10.5935/2446-4775.20160042. | e-ISSN: 2446-4775.

[2] J.A. Silva, M. Bündchen, Conhecimento etnobotânico sobre as plantas medicinais utilizadas pela comunidade do Bairro Cidade Alta, município de Videira, Santa Catarina, Brasil, Unoesc & Ciência - ACBS 2 (2) (2011) 129–140. Available from: https://portalperiodicos.unoesc.edu.br/acbs/article/view/1358. (Accessed 9 January 2020).

[3] L.S. Julião, E. Tavares, C. Lage, S. Leitão, Cromatografia em camada fina de extratos de três quimiotipos de *Lippia alba* (Mill) N.E.Br. (erva-cidreira), Rev. bras. farmacogn., Maringá 13 (supl. 1) (2003) 36–38, https://doi.org/10.1590/S0102-695X2003000300014.

[4] F.F. Barbosa, L.C.A. Barbosa, E.C. Melo, F.M. Botelho, R.H.S. Santos, Influência da temperatura do ar de secagem sobre o teor e a composição química do óleo essencial de *Lippia alba* (Mill) N. E. Brown. Quím, São Paulo, Nova 29 (6) (2006) 1221–1225, https://doi.org/10.1590/S0100-40422006000600014. Dec. 2006.

[5] J.A. Pino Alea, A.G.O. Luiz, R. Baluja, Composición y propiedades antibacterianas del aceite esencial de *Lippia alba* (Mill.) N. E. Brown, Rev Cubana Farm, Ciudad de la Habana 3 (1) (1996). Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75151996000100007&lng=es&nrm=iso. (Accessed 8 January 2020).

[6] A.Q. Silva Júnior, P.L.B. Figueiredo, S.L.F. Sarrazin, L.E.M. Bouillet, R.B. Oliveira, J.G.S. Maia, R.H. Veras Mourão, Seasonal and circadian evaluation of a citral-chemotype from *Lippia alba* essential oil displaying antibacterial activity, Biochem. Systemat. Ecol. 85 (2019) 35–42, https://doi.org/10.1016/j.bse.2019.05.002. ISSN 0305-1978.

[7] H.N.P. Silva, R.N. Souza, E.M.O. Sousa, R.H.V. Mourão, B. Baldisserotto, L.V.F. Silva, Citral chemotype of the *Lippia alba* essential oil as an additive in simulated transport with different loading densities of tambaqui juveniles, Animal Production. Cienc. Rural 50 (4) (2020), https://doi.org/10.1590/0103-8478cr20190815.

[8] F.F. Barbosa, L.C.A. Barbosa, E.C. Melo, F.M. Botelho, R.H.S. Santos, Influência da temperatura do ar de secagem sobre o teor e a composição química do óleo essencial de *Lippia alba* (Mill) N. E. Brown. Quím, Nova, São Paulo 29 (6) (2006) 1221–1225, https://doi.org/10.1590/S0100-40422006000600014. Dec. 2006.

[9] A.C.N. Sobrinho, S.M. Morais, M.M. Marinho, N.V. Souza, D.M. Lima, Antiviral activity on the Zika virus and larvicidal activity on the Aedes spp. of Lippia alba essential oil and β-caryophyllene, Ind. Crop. Prod. 162 (2021) 113281, https://doi.org/10.1016/j.indcrop.2021.113281. ISSN 0926-6690.

[10] I.E.O. Lima, L.A.M. Nascimento, M.S. Silva, Comercialização de plantas medicinais no município de Arapicaca-AL, Rev. Bras. Plantas Med. 18 (2) (2016) 462–472, https://doi.org/10.1590/1983-084X/15_20. Campinas.

[11] C.G. Heldwein, L.L. Silva, E.Z. Gai, C. Roman, T. Parodi, M. Bürger, B. Baldisserotto, E. Flores, B. Heinzmann, S -(+)-Linalool from Lippia alba : sedative and anesthetic for silver catfiHS (Rhamdia quelen), Vet. Anaesth. Analg. 41 (6) (2014) 621–629, https://doi.org/10.1111/vaa.12146.

[12] M.A. Blanco, G.A. Colareda, C. van Baren, A.L. Bandoni, J. Ringuelet, A.E. Consolini, Antispasmodic effects and composition of the essential oils from two South American chemotypes of *Lippia alba*, J. Ethnopharmacol. 149 (3) (2013) 803–809, https://doi.org/10.1016/j.jep.2013.08.007, 7 October 2013.

[13] V.Y. Hatano, A.S. Torricelli, A.C.C. Giassi, L.A. Coslope, M.B. Viana, Anxiolytic effects of repeated treatment with an essential oil from Lippia alba and (R)(-)-carvone in the elevated T-maze, Braz. J. Med. Biol. Res. 45 (2012) 238–243, https://doi.org/10.1590/S0100-879X2012007500021.

[14] M.R. Soto Vasquez, GarcíaPaúl Alvarado, Luis Cerquin, R. Penadillos, W. Sagástegui Guarniz, Composición química y efecto del aceite esencial de las hojas de *Lippia alba* (Verbenaceae) en los niveles de estrés académico de estudiantes universitarios, Arnaldoa, Trujillo 26 (1) (2019) 381–390, https://doi.org/10.22497/arnaldoa.261.26119.

[15] C.M.O. Simões, V. Spitzer, Óleos voláteis, in: C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petrovick (Eds.), Farmacognosia: da planta ao medicamento, UFRGS Ed., 5., Florianópolis/Porto Alegre, 2004, pp. 467–495.

[16] M.G.B. Zoghbi, E.H.A. Andrade, A.S. Santos, M.H.L. Silva, J.G.S. Maia, Essential oils of *Lippia alba* (Mill) N. E. Br growing wild in the brazilian Amazon, Flavour Fragrance J. 13 (1998) 47–48, https://doi.org/10.1002/(SICI)1099-1026(199801/02)13:1<47:AID-FFJ690>3.0.CO;2-0.

[17] M.E. Pascual, K. Slowing, M.E. Carretero, A. Villar, Antiulcerogenic activity of *Lippia alba* (Mill.) NE Brown (Verbenaceae), Il Farmaco 56 (5–7) (2001) 501–504, https://doi.org/10.1016/S0014-827X(01)01086-2.

[18] L.S. Julião, E. Tavares, C. Lage, S. Leitão, Cromatografia em camada fina de extratos de três quimiotipos de *Lippia alba* (Mill) N.E.Br. (erva-cidreira), Rev. bras. farmacogn., Maringá 13 (supl. 1) (2003) 36–38, https://doi.org/10.1590/S0102-695X2003000300014.

[19] C.M.S. Costa, M.G. Cruz, T.B.C. Lima, L.C. Ferreira, A. Ventura, F. Brandão, E. Chagas, F. Chaves, M. Martins, G. Jerônimo, Efficacy of the essential oils of *Mentha piperita, Lippia alba* and *Zingiber officinale* to control the acanthocephalan Neoechinorhynchus buttnerae in Colossoma macropomum, Aquaculture Reports 18 (2020) 100414, https://doi.org/10.1016/j.aqrep.2020.100414. ISSN 2352-5134.

[20] B.N.V. Serra, M.L. Martins, S.A. Pereira, E.C. Chagas, F.C.M. Chaves, M.I.B. Oliveira, G.T. Jerônimo, Ovicidal effect of essential oils of *Lippia alba, Lippia sidoides* and *Lippia gracilis* on the acanthocephalan *Neoechinorhynchus buttnerae* (Eoacanthocephala: neoechinorhynchidae), J FiHS Dis 26 (2022), https://doi.org/10.1111/jfd.13582.

[21] D.R. Oliveira, G.G. Leitão, S.S. Santos, H.R. Bizzo, D. Lopes, C. Alviano, D. Alviano, S. Leitão, Ethnopharmacological study of two Lippia species from Oriximiná, Brazil, J. Ethnopharmacol. 108 (1) (2006) 103–108, https://doi.org/10.1016/j.jep.2006.04.018. ISSN 0378-8741.

[22] L.V.F. Silva, R.H. Veras Mourão, J. Manimala, G.A. Lnenicka, The essential oil of *Lippia alba* and its components affect *Drosophila* behavior and synaptic physiology, J. Exp. Biol. 221 (Pt 14) (2018) jeb176909, https://doi.org/10.1242/jeb.176909.

[23] M.L.F. Barbosa, W.L.C. Ribeiro, J.V. de Araújo Filho, R. de Cássia Alves Pereira, W.P.P. André, A.C.F.L. Melo, D. de Souza Collares Maia Castelo-Branco, S.M. de Morais, L.M.B. de Oliveira, C.M.L. Bevilaqua, In vitro anthelmintic activity of *Lippia alba* essential oil chemotypes against *Haemonchus contortus*, Exp. Parasitol. 244 (2023) 108439, https://doi.org/10.1016/j.exppara.2022.108439.

[24] K. Bonilla-Carvajal, E.E. Stashenko, N. Moreno-Castellanos, Essential oil of carvone chemotype *Lippia alba* (Verbenaceae) regulates lipid mobilization and adipogenesis in adipocytes, Curr. Issues Mol. Biol. 44 (11) (2022) 5741–5755, https://doi.org/10.3390/cimb44110389.

[25] W.L. Quintero, E.M. Moreno, S.M.L. Pinto, S.M. Sanabria, E. Stashenko, L.T. García, Immunomodulatory, trypanocide, and antioxidant properties of essential oil fractions of *Lippia alba* (Verbenaceae), BMC complementary medicine and therapies 21 (1) (2021) 187, https://doi.org/10.1186/s12906-021-03347-6.

[26] T. de Albuquerque Lima, N.M. de Queiroz Baptista, A.P.S. de Oliveira, P.A. da Silva, N.B. de Gusmão, M.T. Dos Santos Correia, T.H. Napoleão, M.V. da Silva, P. M.G. Paiva, 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*, Pestic. Biochem. Physiol. 177 (2021) 104901, https://doi.org/10.1016/j.pestbp.2021.104901.

[27] A.S. Borges, C.M.S. Bastos, D.M. Dantas, C.G.B. Milfont, G.M.H. Brito, L. Pereira-de-Morais, G.A. Delmondes, R.E.R. da Silva, E. Kennedy-Feitosa, F.P.A. Maia, C. M.G. Lima, T. Bin Emran, H.D.M. Coutinho, I.R.A. Menezes, M.R. Kerntopf, G. Caruso, R. Barbosa, Effect of *Lippia alba* (Mill.) N.E. Brown essential oil on the human umbilical artery, Plants 11 (21) (2022) 3002, https://doi.org/10.3390/plants11213002.

[28] B.N.V. Serra, M.L. Martins, S.A. Pereira, E.C. Chagas, F.C.M. Chaves, M.I.B. de Oliveira, G.T. Jerônimo, Ovicidal effect of essential oils of *Lippia alba, Lippia sidoides* and *Lippia gracilis* on the acanthocephalan Neoechinorhynchus buttnerae (Eoacanthocephala: neoechinorhynchidae), J. Fish. Dis. 45 (4) (2022) 569–577, https://doi.org/10.1111/jfd.13582.

[29] L.F. Postay, D.S. Cabral, O.A. Heringer, L.V. Vieira, L.R. de Moraes, G. Freitas, L.C. Gomes, 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, Environ. Sci. Pollut. Res. Int. 28 (8) (2021) 10224–10233, https://doi.org/10.1007/s11356-020-11483-8.

[30] D.S. Gomes, D.A. Negrão-Corrêa, G.S. Miranda, J.G.M. Rodrigues, T.J.F.L. Guedes, W. de Lucca Junior, J.C.F. Sá Filho, D.A.C. Nizio, A.F. Blank, V.L.C. Feitosa, S. S. Dolabella, *Lippia alba* and *Lippia gracilis* essential oils affect the viability and oviposition of *Schistosoma mansoni*, Acta Trop. 231 (2022) 106434, https://doi.org/10.1016/j.actatropica.2022.106434.

[31] L.G.A.D. Santos Filho, R.B.D. Reis, A.S.Q. Souza, K.M. Canuto, E.S. Brito, K.N.C. Castro, A.M.L. Pereira, F.M. Diniz, Chemical composition and biological activities of the essential oils from *Lippia alba* and *Lippia origanoides*, An. Acad. Bras. Cienc. 95 (1) (2023) e20220359, https://doi.org/10.1590/0001-3765202320220359.

[32] C.L. de Lima, R.D. Morales-Gamba, T.S. Malcher Neto, J.F.M. Barcellos, B.M. Heinzmann, D. Schmidt, B. Baldisserotto, J.L. Marcon, Eugenol and *Lippia alba* essential oils as effective anesthetics for the Amazonian freshwater stingray *Potamotrygon wallacei* (Chondrichthyes, Potamotrygonidae), Fish Physiol. Biochem. 47 (6) (2021) 2101–2120, https://doi.org/10.1007/s10695-021-01029-1.

[33] M.C. Pagotti, A.C.B.B. Candido, M.G. Marçal, T.M. Vieira, M. Groppo, M.L.A. Silva, D.S. Ferreira, V.R. Esperandim, A.E.M. Crotti, L.G. Magalhães, Trypanocidal activity of *Dysphania ambrosioides, Lippia alba*, and *Tetradenia riparia* essential oils against *Trypanosoma cruzi*, Chem. Biodivers. 18 (12) (2021) e2100678, https://doi.org/10.1002/cbdv.202100678.

[34] C. de Fatima Alves Nonato, C.J. Camilo, D.O. Duarte Leite, M.G. Lúcio Albuquerque da Nobrega, J. Ribeiro-Filho, I.R. Alencar de Menezes, J.F. Tavares, J. G. Martins da Costa, Comparative analysis of chemical profiles and antioxidant activities of essential oils obtained from species of *Lippia* L. by chemometrics, Food Chem. 384 (2022) 132614, https://doi.org/10.1016/j.foodchem.2022.132614.

[35] G.A. de Brito, P.F. Rocha de Oliveira, C.M. de Andrade Silva, M.F. de Araújo Neto, F.H.A. Leite, P.R.R. Mesquita, T.F. Mota, J.T. Magalhães-Junior, Identification of bioactive compounds against *Aedes aegypti* (Diptera: Culicidae) by bioassays and in silico assays, Chem. Biodivers. 18 (9) (2021) e2100242, https://doi.org/10.1002/cbdv.202100242.

[36] M.A. Tabari, A. Rostami, A. Khodashenas, F. Maggi, R. Petrelli, C. Giordani, L.A. Tapondjou, F. Papa, Y. Zuo, K. Cianfaglione, M.R. Youssefi, Acaricidal activity, mode of action, and persistent efficacy of selected essential oils on the poultry red mite (*Dermanyssus gallinae*), Food Chem. Toxicol. : an international journal published for the British Industrial Biological Research Association 138 (2020) 111207, https://doi.org/10.1016/j.fct.2020.111207.

[37] M.T.S. Trevisan, F.V.V. Macedo, M. van de Meent, I.K. Rhee, R. Verpoort, Seleção de plantas com atividade anticolinesterase para tratamento da doença de Alzheimer. Quím, Nova, São Paulo 26 (3) (2003) 301–304, https://doi.org/10.1590/S0100-40422003000300002.

[38] A.S. Santos, S.M. Alves, F.J.C. Figueiredo, O.G.R. Neto, Comunicado técnico: Descrição de sistema e de métodos de extração de óleos essenciais e determinação de umidade de biomassa em laboratório. Ministério da Agricultura, Pecuária e Abastecimento (2004). Disponível em: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/402448. (Accessed 26 May 2020).

[39] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured PubliHSing. Carol Stream. IL, 4 ed., 2007. USA.

[40] L. Mondello, FFNSC 2: Flavors and Fragrances of Natural and Synthetic Compounds, Mass Spectral Database, John Wiley & Sons Inc., New York, 2011, 2011.

[41] A. Marston, J.E. Kissling, K. Hostettmann, Um método bioautográfico rápido por TLC para a detecção de inibidores de acetilcolinesterase e butirilcolinesterase em plantas, Phytochem. Anal. 13 (2002) 51–54, https://doi.org/10.1002/pca.623.

[42] G.L. Ellman, K. Courtney, V. Andres, R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95, https://doi.org/10.1016/0006-2952(61)90145-9.

[43] S.M. Albano, A.S. Lima, G.M. Miguel, L.G. Pedro, J.G. Barroso, A.C. Figueiredo, Antioxidant, anti-5-lipoxygenase and antiacetylcholinesterase activities of essential oils and decoction waters of some aromatic plants, Record Nat. Prod. 6 (1) (2012) 35–48, 2012.

[44] K.A. Wojtunik-Kulesza, K. Targowska-Duda, K. Klimek, G. Ginalska, K. Jóźwiak, M. Waksmundzka-Hajnos, Ł. Cieśla, Volatile terpenoids as potential drug leads in Alzheimer's disease, Open Chem. 15 (2017) 332–343, https://doi.org/10.1515/chem-2017-0040.

[45] Jonah Cheung, Michael J. Rudolph, Fiana Burshteyn, Michael S. Cassidy, Ebony N. Gary, James Love, Matthew C. Franklin, Jude J. Height, Structures of human acetylcholinesterase in complex with pharmacologically important ligands, J. Med. Chem. 55 (22) (2012) 10282–10286, https://doi.org/10.1021/jm300871x.

[46] Jiansong Fang, Ping Wu, Ranyao Yang, Li Gao, Li Chao, Dongmei Wang, Song Wu, Lin Liu Ai, Guan-Hua Du, Inhibition of acetylcholinesterase by two genistein derivatives: kinetic analysis, molecular docking and molecular dynamics simulation, Acta Pharm. Sin. B 4 (6) (2014) 430–437, https://doi.org/10.1016/j.apsb.2014.10.002. ISSN 2211-3835.

[47] I.A. Guedes, L.S.C. Costa, K.B. Dos Santos, A.L.M. Karl, G.K. Rocha, I.M. Teixeira, M.M. Galheigo, V. Medeiros, E. Krempser, F.L. Custódio, H.J.C. Barbosa, M. F. Nicolás, L.E. Dardenne, Drug design and repurposing with DockThor-VS web server focusing on SARS-CoV-2 therapeutic targets and their non-synonym variants, Sci. Rep. 11 (1) (2021) 5543, https://doi.org/10.1038/s41598-021-84700-0.

[48] Giulio Poli, Adriano Martinelli, Tiziano Tuccinardi, Reliability analysis and optimization of the consensus docking approach for the development of virtual screening studies, J. Enzym. Inhib. Med. Chem. 31 (sup2) (2016) 167–173, https://doi.org/10.1080/14756366.2016.1193736.
[49] D. Barak, A. Ordentlich, D. Stein, Q.S. Yu, N.H. Greig, A. Shafferman, Accommodation of physostigmine and its analogues by acetylcholinesterase is dominated by hydrophobic interactions, Biochem. J. 417 (1) (2009) 213–222, https://doi.org/10.1042/BJ20081276.
[50] K.A. Wojtunik-Kulesza, K. Targowska-Duda, K. Klimek, G. Ginalska, K. Jóźwiak, M. Waksmundzka-Hajnos, Ł. Cieśla, Volatile terpenoids as potential drug leads in Alzheimer's disease, Open Chem. 15 (2017) 332–343, https://doi.org/10.1515/chem-2017-0040.
[51] D.C.S. Barbosa, et al., Chemical composition and acetylcholinesterase inhibitory potential, *in silico*, of *Myrciaria floribunda* (H. West ex Willd.) O. Berg fruit peel essential oil, Ind. Crop. Prod. 151 (2020) 112372, https://doi.org/10.1016/j.indcrop.2020.112372, 1 September.
[52] S. Savelev, E. Okello, N.S. Perry, R.M. Wilkins, E.K. Perry, Synergistic and antagonistic interactions of anticholinesterase terpenoids in Salvia lavandulaefolia essential oil, Pharmacol. Biochem. Behav. 75 (3) (2003) 661–668, https://doi.org/10.1016/s0091-3057(03)00125-4.
[53] M.I. Picollo, A.C. Toloza, G. Mougabure Cueto, J. Zygadlo, E. Zerba, Anticholinesterase and pediculicidal activities of monoterpenoids, Fitoterapia 79 (4) (2008) 271–278, https://doi.org/10.1016/j.fitote.2008.01.005.
[54] W.M.N.H.W. Salleh, S. Khamis, M.A. Nafiah, S.A. Abed, Chemical composition and anticholinesterase inhibitory activity of the essential oil of *Pseuduvaria macrophylla* (Oliv.) Merr. from Malaysia, Nat. Prod. Res. 35 (11) (2021) 1887–1892, https://doi.org/10.1080/14786419.2019.1639183.
[55] W. Chaiyana, S. Okonogi, Inhibition of cholinesterase by essential oil from food plant, Phytomedicine 19 (2012) 836–839.
[56] Z. Aumeeruddy-Elalfi, N. Lall, B. Fibrich, A. Blom Van Staden, M. Hosenally, M.F. Mahomoodally, Selected essential oils inhibit key physiological enzymes and possess intracellular and extracellular antimelanogenic properties in vitro, J. Food Drug Anal. 26 (1) (2018) 232–243, https://doi.org/10.1016/j.jfda.2017.03.002.
[57] M.D. López, M.J. Pascual-Villalobos, Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control, Ind. Crop. Prod. 31 (2010) 284–288.
[58] M. Awasthi, A.K. Upadhyay, S. Singh, V.P. Pandey, U.N. Dwivedi, Terpenoids as promising therapeutic molecules against Alzheimer's disease: amyloid beta- and acetylcholinesterase-directed pharmacokinetic and molecular docking analyses, Mol. Simulat. 44 (1) (2017) 1–11, https://doi.org/10.1080/08927022.2017.1334880.
[59] S.M. Morais, A. C. Nogueira Sobrinho, H.R. Liberato, R.C.A. Pereira, C. Pessoa, D.R. Alves, R.O.S. Fontenelle, Biotechnological potential of essential oils from different chemotypes of Lippia alba (Mill.) N.E.Br. ex Britton & P. Wilson, Bol Latinoam Caribe Plant Med Aromat 21 (6) (2022) 725–736, https://doi.org/10.37360/blacpma.22.21.6.44.