

Evaluation of *Bauhinia unguolata* Essential Oil as a New Acetylcholinesterase Inhibitor from an *in silico* and *in vitro* Perspective in the Northern Amazon of Brazil

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Abstract: The *Bauhinia unguolata*, also known by its common name “pata de vaca”, is one of the species used in Brazil for medicinal purposes, and is commonly used for the treatment of diabetes. In this study, the authors studied the interaction between the chemical constituents which are present in the essential oil of *Bauhinia unguolata* (EOBU), collected in Boa Vista-RR, Legal Amazon, and their effects on the enzyme acetylcholinesterase (AChE) in the essential oil. The analysis that we perform includes proton magnetic resonance (¹H NMR), enzymatic inhibition, molecular docking, *in silico* toxicity prediction, enrichment analysis, and target prediction for biological interactions. According to the tests performed on the essential oil, it obtained 100% inhibition of the enzyme AChE. During ¹H NMR experiments, it was found that α -Bisabolol, one of the main components, had a significant alteration in its chemical shift. A molecular docking analysis confirmed that this compound binds to the AChE enzyme, which confirms the ¹H NMR analysis. The results of this work showed that the major component of EOBU acted as a possible inhibitor of AChE enzyme *in vitro* and *in silico* assays. These results show that EOBU could be potentially applied in Alzheimer's disease treatment.

Key words: α -Bisabolol, Alzheimer, essential oil, acetylcholinesterase inhibitors, neurodegenerative diseases

1 Introduction

Neurodegenerative diseases are characterized by irreversible damage to specific neurons, leading to gradual loss and impairment of certain nervous system functions. Some are now considered the biggest causes of dementia global-

ly¹⁾. Alzheimer's disease (AD) is a pathology that currently represents the most frequent form of age-related dementia, with 2-10% of those affected under 65 years old and about 30-50% over 85. Worldwide, about 50 million people live with AD, and estimates predict that, by the year 2050,

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around 150 million people will be affected by the disease^{2,3)}. According to Wimo *et al.*, the global cost of 2019 for dementia was over 1 trillion dollars, estimated that around 800 billion were spent on AD alone, according to surveys for the year 2015. Most people who have dementia live in low- or middle-income countries (61%); however, due to the high costs of having a good structure in health systems for these patients, these countries are also the ones that spend less on these types of treatment⁴⁾.

The mechanisms involved in the evolution of the pathology have not yet been fully unraveled. Nevertheless, three striking pathophysiological characteristics are observed in patients with AD, which are: intracellular, formed mainly by the tau protein, as well as gradual neurodegeneration⁵⁾.

The cholinergic hypothesis of Alzheimer's disease is related to the ability of drugs that stimulate central cholinergic function to induce an improvement in cognitive function and some effects resulting from the disease. The use of these cholinesterase inhibitors (I-ChE) is the primary drug currently licensed for the specific treatment of AD. The Food and Drug Administration (FDA) has approved several drugs on the market today that can reduce the effects of the disease, such as Tacrine, Donepezil, Physostigmine, Rivastigmine, Huperzine A and Galanthamine^{6,7)}. However, some of these can cause undesirable effects on patients undergoing treatment, such as gastrointestinal: nausea, vomiting, diarrhea, anorexia, dyspepsia, abdominal pain, increased acid secretion, cardiovascular: fluctuating blood pressure, syncope, arrhythmia, bradycardia; dizziness, headache, agitation, insomnia, cramps, sweating and increased bronchial secretion. Furthermore, these drugs only delay cognitive symptoms, and about half of those who take these drugs do not respond to them^{8,9)}.

Essential oils have the function of protecting the plant against attacks by predators, such as insects and microorganisms, as well as attracting pollinators for the dissemination of pollen and seeds. EOs are mainly composed of substances such as fatty acid esters, mono, and sesquiterpene phenylpropanoids. Their chemical components give them aroma and color characteristics¹⁰⁾. Essential oils are stored in secretory, epidermal cells, cavities, channels or glandular trichomes in all plant organs and have been used since ancient times for having biological properties such as anti-inflammatory, antibacterial, antifungal, antioxidant, and antiviral^{11–13)}, and some essential oils and their constituents, such as sesquiterpenes, have been investigated for their potential neuroprotective effects^{14–16)}.

The species *Bauhinia unguolata* which in Latin means hoof or foot of ox, presents itself as a wild, small tree that has a rigid stem with fibrous bark and leaves that form two lobes whose design resembles the trail of a cow's paw with pointed slits characteristic of species of Bauhinias, and the flowers are white and the seeds dark brown. It is commonly found in the Amazon region and Northeast of Brazil and is

more recognized for its hypoglycemic activity. Further studies also reveal that extracts of this plant decrease cholesterol and triglyceride levels^{17,18)}. Studies on the chemical constitution of *B. unguolata* have revealed the presence of different flavonoids in its leaves, such as quercetin, kaempferol, naringenin, liquiritigenin, eridictiol^{19,20)}. These flavonoids have antioxidant, anti-inflammatory, antithrombotic, antiallergic and hypoglycemic activities²¹⁾.

Oil essential of *B. unguolata* (EOBU) can be a promising natural biological agent in the fight against AD due to its inhibitory action on the enzyme acetylcholinesterase (AChE)^{22,23)}. Therefore, the present research focused on highlighting the chemical profile and interaction of substances present in the EOBU concerning its possible inhibitory effect on the enzyme acetylcholinesterase as an alternative in the treatment of AD.

2 Materials and Methods

2.1 Obtaining the leaves of *Bauhinia unguolata*

The medicinal plant *B. unguolata* was collected during the rainy season (July to September 2020) in Boa Vista, Roraima, Amazônia, Brazil. The plant's georeferences indicate that it was located at 2° 51' 11.03976 N, 60° N latitude, and 60° 38' 26 longitude W. Dr. Amélia Tuler identified one specimen of the plant, which has been listed in the Herbarium of the Federal University of Roraima (UFRR) as number 9685. This specimen has also been registered in SISGEN under the number A789974.

2.2 Essential oil extraction

Plant material was collected and washed with distilled water. The EOBU was obtained by hydrodistillation using a Clevenger apparatus with a double condenser of Spell's model for an uninterrupted 2-hour distillation period. The fresh leaves were cut into approximately 1 cm⁻¹ and subsequently added to 1000 g in a 12 L flask with 6 L of distilled water. The flask was heated in a heating mantle at 100 ± 2°C. At the end of the process, the water was removed by adding anhydrous sodium sulfate and subsequently stored in a freezer at -20 ± 2°C²⁴⁾.

2.3 Enzymatic activity

Enzymatic analysis, it was used the Commercial Acetylcholinesterase (AChE) from *Electrophorus electricus* electric organ (Type VI-S), acetylthiocholine iodide, bovine serum albumin, 5,50-dithiobis (2-nitrobenzoic acid) (DTNB), tris(hydroxymethyl)aminomethane were purchased from Sigma-Aldrich (St. Louis, MO, EUA).

2.3.1 Acetylcholinesterase enzyme inhibition

To determine enzymatic activity, assays were performed on microplates in quadruplicate. An aliquot of 200 µL of the chromogenic reagent (DTNB 0.25 mmol.L⁻¹ in Tris-HCl

0.5 mol.L⁻¹ pH 7.4) and 20 µL of commercial AChE enzyme (*E. electricus*) 0.67 mg.mL⁻¹) were added. After incubation for 1 h, 20 µL of 62 mM acetylthiocholine iodide was added before reading and the degradation of this substrate was measured spectrophotometrically after 180 s at 405 nm. Activity (U) was defined as the amount of enzyme capable of converting 1 µmol of substrate per minute. Protein was determined by the Bradford method²⁵⁾, adapted by Sedmak & Grossberg²⁶⁾. For selective inhibition, AChE was exposed to EOBU, incubating 10 µL of commercial AChE enzyme with 10 µL of EOBU (at six concentrations ranging from 2.4×10^{-2} to 2.4×10^{-7} mol.L⁻¹ of the major compound), for 1 h according to the methodology described above, and the residual activity was calculated using the absence of essential oil as 100% enzymatic activity. Results were expressed as percent inhibition (mean ± SD) versus EOBU major compound concentration to estimate IC₂₀ and IC₅₀ (concentrations at which enzymatic activity is inhibited by 20 and 50%, respectively).

2.4 Proton nuclear magnetic resonance (¹H NMR)

¹H NMR titration studies were carried out to investigate the interaction between the constituents of EOBU and AChE. A dispersion of 700 µL of the EOBU dissolved in DMSO-d₆ was prepared and titrated with AChE solution, obtaining final concentrations ranging from 5 to 20 µL.mL⁻¹. After adding the enzyme, the ¹H NMR spectra were recorded, and the chemical shift of protons was documented. To avoid interference from the solvent in the analysis performed, the final concentration of PBS solution in added d-DMSO was 3%, proportions commonly used in ligand-protein interaction experiments.

2.5 Computational analysis

2.5.1 *In silico* molecular docking

The EOBU molecules were obtained from PubChem²⁷⁾ with the respective identifiers: α-Cadinene (CID: 12306048), α-Bisabolol (CID: 1549992), α-Bisabolol Oxide A (CID: 6431314), α-Bisabolol Oxide B (CID: 6432283), α-Humulene (CID: 5281520), α-Pinene (CID: 440968), α-Thujene (CID: 637518), Aromadendrene (CID: 91354), β-Caryophyllene (CID: 5281515), β-Elementene (CID: 9859094), β-Pinene (CID: 440967), Camphene (CID: 6616), Copaene (CID: 12303902), Cubenol (CID: 11770062), δ-Cadinene (CID: 441005), Spathulenol (CID: 92231), Eucalyptol (CID: 2758), Epi-γ-Eudesmol (CID: 6428429), γ-Elementene (CID: 6432312), γ-Murolene (CID: 12313020), Linalool (CID: 6549), Nerolidol (CID: 5284507), Vanlencene (CID: 9855795), (2E,6Z)-farnesol (CID: 1549109), Caryophyllene Oxide (CID: 1742210), Donepezil (CID: 3152), Galantamine (CID: 9651), Huperzine A (CID: 449069), Neostigmine (CID: 4456), Rivastigmine (CID: 77991), Physostigmine (CID: 5983), Pyridostigmine (CID: 4991) and Tacrine (CID: 1935). These last eight molecules were used

as positive controls for binding to AChEs since they are FDA-approved drugs.

The human AChE three-dimensional structure was obtained from the Protein Data Bank (ID: 4EY7)²⁸⁾, with a resolution of 2.35 Å. *E. electricus* AChE was modelled through SWISS-MODEL²⁹⁾ web server (<https://swissmodel.expasy.org>) using 4BDT³⁰⁾ from PDB as a template and validated it through Verify3D (Supplementary Materials), due to its absent tridimensional structure in the PDB database. To find out which binding sites would be used for molecular docking, we carried out an analysis using GRASP software, a program based on machine learning that searches for binding sites by calculating the accessibility of residues to the solvent, physicochemical characteristics, and established interactions³¹⁾.

Nevertheless, we observed that the analysis of the GRASP binding site was compatible with results found in the literature, for the human structure and the template used in the *E. electricus* model^{30, 32)}. The molecular docking tests were performed in the Autodock Vina software and the adjustment of their respective parameters, such as water removal and addition of polar hydrogens, on the Autodock tools v. 1-5-7³³⁾. During the preparation of AChE for docking, all water molecules were removed from the medium, polar hydrogens were added to the amino acids, and the Kollman charges were added to the proteins. The defined grid box for the human AChE had a size of 34 × 34 × 34 with a spacing of 0.375 Å, while the center of mass was -14.753, -43.913, 29.561 on the chain A. The defined grid box for the human AChE had a size of 34 × 34 × 34 with a spacing of 0.375 Å, while the center of mass was -4.746, -39.227, and -56.271 on the chain A. To validate molecular docking results, a redocking using GOLD software RMS analysis was applied (Supplementary Materials). The molecular interactions between the ligands and the amino acids were demonstrated and analyzed using BIOVIA Discovery Studio Visualizer 2021 and Pymol 2.5.

2.5.2 Pharmacokinetic parameters prediction

The analysis of ADME (Absorption, Distribution, Metabolism, and Excretion) parameters was performed using the SwissADME web server for the molecules present in the EOBU³⁴⁾.

2.5.3 Drug-target interaction prediction and enrichment analysis

The drug-target interaction predictions were performed on Swiss Target Prediction for the species, *Homo sapiens*³⁵⁾. The predicted targets were submitted to Gene Ontology (GO) and Pathway enrichment analysis through FunRich functional annotation software and ShinyGO, respectively^{36, 37)}.

2.6 Statistical analysis

All data was expressed as mean and standard deviation. Statistical analysis performed by Student's Teste T e

ANOVA followed by the Two-tailed, Tukey-Kramer test and Bonferroni ($p < 0.05$) using GraphPad Prism 8 Software.

3 Results and Discussion

3.1 Enzymatic inhibition

Some natural chemical compounds inhibit the enzymatic activity of AChE, making the investigation of these compounds extremely important for the development of new targeted drugs for AD. According to Mota *et al.* (2012)³⁸, in their *in vitro* study with plant extracts, they considered a weak AChE inhibition values lower than 30%, results obtained for the species *Maytenus rigida* (percentage of inhibition of 7%) and *Hyptis fruticosa* (percentage of inhibition of 11%), values between 30 and 50% were considered moderate inhibition, percentages obtained for the species *P. piperoides* (40%) and *Moringa oleifera* (47%) and potent values of inhibition above 50%, which was the result obtained for the extract of *V. agnus-castus* L. which inhibited 74% of AChE activity. Among the species studied by Mota *et al.* was *Bauhinia cheilantha*, which belongs to the same genus as the species under study, did not show inhibitory activity³⁸. These results differ from this research, as the species *B. unguolata*, the target of our study, inhibited the activity of the AChE enzyme by 100% (Fig. 1). The IC_{20} and IC_{50} values of EOBU shown in this work were lower than those of Neostigmine (IC_{20} 2.61×10^{-8} and 2.30×10^{-5}), a commercial AChE inhibitor, showing that the major compound of EOBU has a greater inhibition po-

tential from AChE.

AChE inhibition prevents the degradation of the brain neurotransmitter ACh, increasing the defects in brain cholinergic transmission that occur mainly in neurodegenerative disorders as the disease progresses⁷. Since the activity of human AChE is strongly related to the physiological state, it is essential to explore new enzymatic inhibitors that allow the development and application of new therapeutic methods.

3.2 Computational analysis

3.2.1 Molecular docking

The molecular docking study was used to structurally examine the binding interactions of EOBU compounds with the AChE enzyme at its active site. Besides, it drew comparisons with anticholinesterase molecules used by patients with disorders of the cholinergic system. A second docking between EOBU content to the *E. electricus* AChE was also performed to compare results between species.

The α -Bisabolol, α -Bisabolol Oxide A, α -Bisabolol oxide B, β -Caryophyllene, and Epi- γ -eudesmol molecules, that are the predominant components of EOBU, showed excellent efficiency of fitting with human AChE (Table 1), obtaining values of -8.7 , -9.5 , -9.1 , -8.4 , and -9.2 kcal.mol⁻¹, respectively. Their results demonstrated with the *E. electricus* AChE (Table 2) were: -8.1 , -7.7 , -9.0 , -8.0 , and -6.8 kcal.mol⁻¹ for, respectively, α -Bisabolol, α -Bisabolol Oxide A, α -Bisabolol Oxide B, β -Caryophyllene, and Epi- γ -eudesmol. This indicates that the EOBU has a greater affinity for the human form of AChE and inhibits it

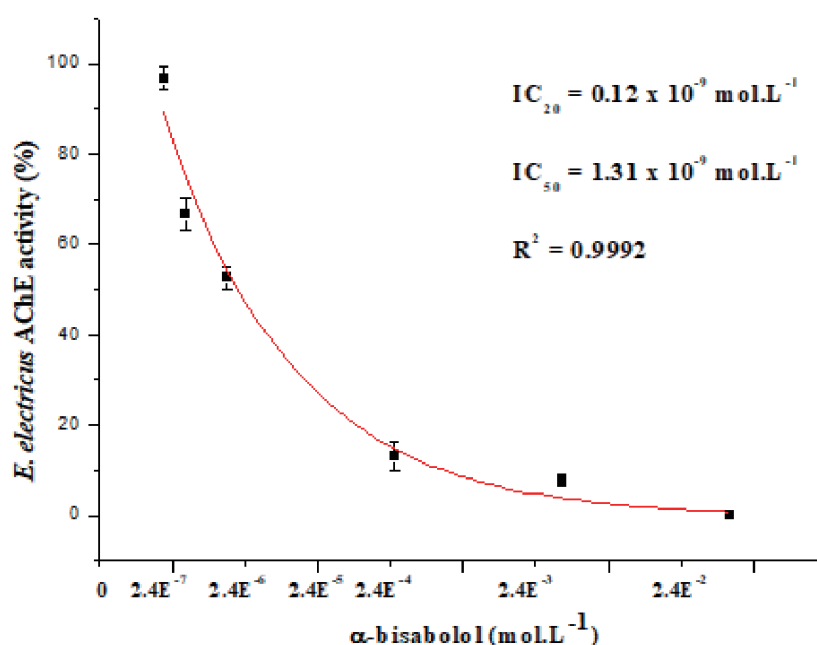


Fig. 1 AChE enzyme inhibition plot. Effect of α -Bisabolol main component of EOBU on AChE enzyme. Data are expressed as mean $\pm$ SD. IC_{50} value = 1.31×10^{-9} mol.L⁻¹ and IC_{20} = 0.12×10^{-9} mol.L⁻¹. All assays were performed at 25°C.

Table 1 Lowest binding energy in kcal.mol⁻¹ of each ligand to the human acetylcholinesterase.

Molecules	Binding energy (kcal.mol ⁻¹)	Molecules	Binding energy (kcal.mol ⁻¹)
Aromadendrene	- 8.8	α-Pinene	- 6.9
Camphene	- 6.5	α-Thujene	- 6.8
Caryophyllene Oxide	- 8.6	β-Caryophyllene	- 8.4
Copaene	- 8.6	β-Elemene	- 8.1
Cubenol	- 8.7	β-Pinene	- 6.9
Epi-γ-eudesmol	- 9.2	γ-Elemene	- 7.9
Eucalyptol	- 6.9	γ-Murolene	- 9.0
Farnesol	- 8.6	δ-Cadinene	- 9.1
Linalool	- 6.5	Donepezil ^a	- 11.8
Nerolidol	- 8.3	Galanthamine ^a	- 10.5
Spathulenol	- 8.4	Huperzine A ^a	- 9.9
Valencene	- 8.8	Neostigmine ^a	- 7.4
α-Bisabolol	- 8.7	Physostigmine ^a	- 9.1
α-Bisabolol Oxide A	- 9.5	Pyridostigmine ^a	- 6.3
α-Bisabolol Oxide B	- 9.1	Rivastigmine ^a	- 8.1
α-Cadinene	- 9.1	Tacrine ^a	- 8.8
α-Humulene	- 7.9		

^aAcetylcholinesterase inhibitors drugs approved by the FDA.

Table 2 Lowest binding energy in kcal.mol⁻¹ of each ligand to the *E. electricus* acetylcholinesterase.

Molecules	Binding energy (kcal.mol ⁻¹)	Molecules	Binding energy (kcal.mol ⁻¹)
Aromadendrene	- 8.8	α-Pinene	- 6.0
Camphene	- 6.3	α-Thujene	- 6.6
Caryophyllene Oxide	- 8.1	β-Caryophyllene	- 8.0
Copaene	- 7.8	β-Elemene	- 8.0
Cubenol	- 8.6	β-Pinene	- 6.4
Epi-γ-eudesmol	- 6.8	γ-Elemene	- 8.0
Eucalyptol	- 6.2	γ-Murolene	- 8.2
Farnesol	- 7.3	δ-Cadinene	- 9.1
Linalool	- 6.4	Donepezil ^a	- 7.8
Nerolidol	- 7.5	Galanthamine ^a	- 8.5
Spathulenol	- 8.3	Huperzine A ^a	- 10.1
Valencene	- 8.7	Neostigmine ^a	- 7.5
α-Bisabolol	- 8.1	Physostigmine ^a	- 8.3
α-Bisabolol Oxide A	- 7.7	Pyridostigmine ^a	- 6.7
α-Bisabolol Oxide B	- 9.0	Rivastigmine ^a	- 7.6
α-Cadinene	- 9.2	Tacrine ^a	- 9.5
α-Humulene	- 7.7		

^aAcetylcholinesterase inhibitors drugs approved by the FDA.

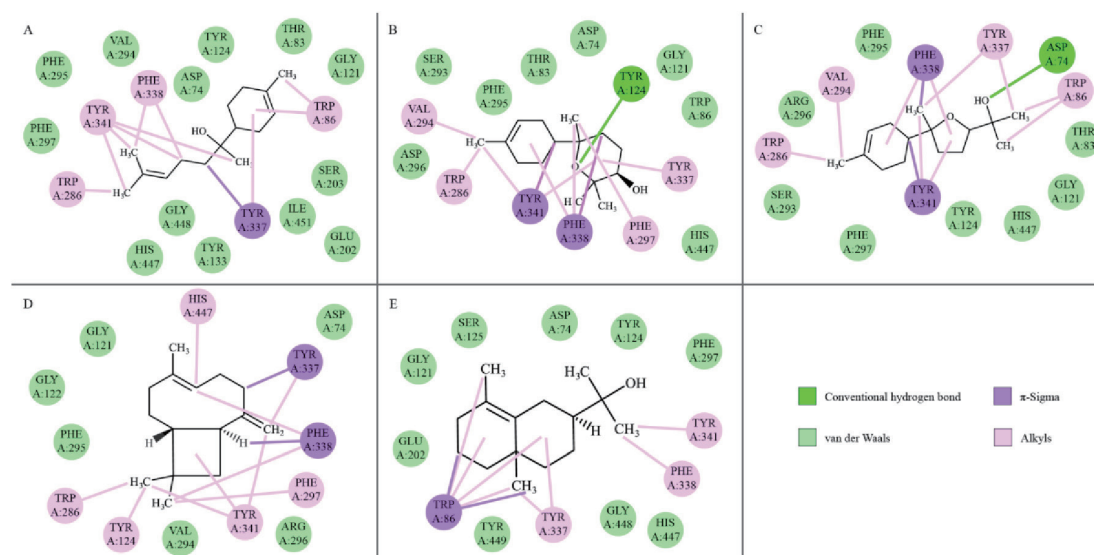


Fig. 2 Structures represent the interaction between the residues of the binding site and the A, B, C, D, and E represent the interactions between the residues of the binding site of human AChE and the ligands α -Bisabolol (A), α -Bisabolol Oxide A (B), α -Bisabolol Oxide B (C), β -Caryophyllene (D), and Epi- γ -Eudesmol (E).

to a greater extent.

EOBU components exhibited similar amino acid interactions with human AChE (Fig. 2), with Asp-74, Gly-121, Tyr-124, Phe-297, Tyr-337, Phe-338, Tyr-341, and His-447 being the most commonly interacted residues. α -Bisabolol (Fig. 2a) interacted with the active site of the human AChE through Asp-74, Thr-83, Trp-86, Gly-121, Tyr-124, Tyr-133, Glu-202, Ser-203, Trp-286, Phe-295, Val-294, Phe-297, Tyr-337, Phe-338, Tyr-341, His-447, Gly-448, and Ile-451, where Alkyl, van der Waals, and π -sigma interactions were observed. α -Bisabolol Oxide A (Fig. 2b) showed interactions between Asp-74, Thr-83, Trp-86, Gly-121, Tyr-124, Trp-286, Ser-293, Val-294, Phe-295, Asp-296, Phe-297, Tyr-337, Phe-338, Tyr-341, and His-447. α -Bisabolol Oxide B (Fig. 2c) between Asp-74, Thr-83, Trp-86, Gly-121, Tyr-124, Trp-286, Ser-293, Val-294, Phe-295, Arg-296, Phe-297, Tyr-337, Phe-338, Tyr-341, and His-447. β -Caryophyllene (Fig. 2d) between Asp-74, Gly-121, Gly-122, Tyr-124, Trp-286, Val-294, Phe-295, Arg-296, Phe-297, Tyr-337, Phe-338, Tyr-341, and His-447. Epi- γ -eudesmol (Fig. 2e) showed structural interactions with the molecule through the amino acids Asp-74, Trp-86, Gly-121, Tyr-124, Ser-125, Glu-202, Phe-297, Tyr-337, Phe-338, Tyr-341, His-447, Gly-448, and Tyr-449.

EOBU components exhibited similar amino acid interactions with *E. electricus* AChE (Fig. 3), with Trp-108, Tyr-146, and Tyr-355 being the most commonly interacted residue. α -Bisabolol (Fig. 3a) bonds with the enzymatic active site through Gly-104, Thr-105, Trp-108, Trp-108, Tyr-141, Gly-142, Gly-143, Ser-147, Gly-148, Ser-149, Leu-152, Tyr-155, Glu-224, Ser-225, Tyr-355, Trp-486, Ile-493, Gly-495, Tyr-496, and Ile-498, with Alkyl, van der Waals,

carbon hydrogen bond and π -sigma interactions. α -Bisabolol Oxide A (Fig. 3b) interacted with Asp-96, Trp-108, Gly-142, Gly-143, Tyr-146, Ser-147, Gly-148, Tyr-155, Tyr-355, Trp-486, Ile-493, His-494, Tyr-496, and Gly-495. α -Bisabolol Oxide B (Fig. 3c) formed interactions between Gly-104, Thr-105, Trp-108, Gly-143, Gly-144, Tyr-146, Ser-147, Glu-224, Ala-226, Ser-255, Phe-315, Phe-356, Trp-486, Ile-493, His-494, Gly-495, and Tyr-496. β -Caryophyllene (Fig. 3d) showed interactions between Tyr-94, Asp-96, Ser-98, Tyr-146, Trp-304, Leu-307, Gly-311, Leu-312, Phe-313, Arg-314, Phe-315, Tyr-355, Phe-356, and Tyr-359. Epi- γ -eudesmol (Fig. 3e) established interactions to *E. electricus* AChE through Tyr-94, Asp-96, Tyr-146, Trp-304, Leu-307, Gly-311, Leu-312, Phe-313, Phe-314, Phe-315, Tyr-355, and Phe-356.

Most interactions were mediated by alkyl and π -sigma bonds, while hydrogen bonds were only seen in α -Bisabolol Oxide A and B. In general, *in silico* molecular docking analysis coincides with *in vitro* analysis where in human cells of the N2a lineage a significant reduction in AChE activity and the ability to prevent ACh depletion was observed, in addition to researchers observing A β peptide anti-aggregating activities and anti-apoptotic properties in α -bisabolol-treated PC12 lineage cells^{39, 40}. When compared to the positive control group, the molecules that make up the EOBU present close-fitting efficiency values, where Donepezil (−11.8 and −7.8 kcal.mol^{−1} for human and *E. electricus* AChE, respectively), Galantamine (−10.5 and −8.5 kcal.mol^{−1}), Huperzine A (−9.9 and −10.1 kcal.mol^{−1}), Physostigmine (−9.1 and −8.3 kcal.mol^{−1}), Tacrine (−8.8 and −9.5 kcal.mol^{−1}), Rivastigmine (−8.1 and −7.6 kcal.mol^{−1}), Neostigmine (−7.4 and −7.5 kcal.mol^{−1}), and Pyr-

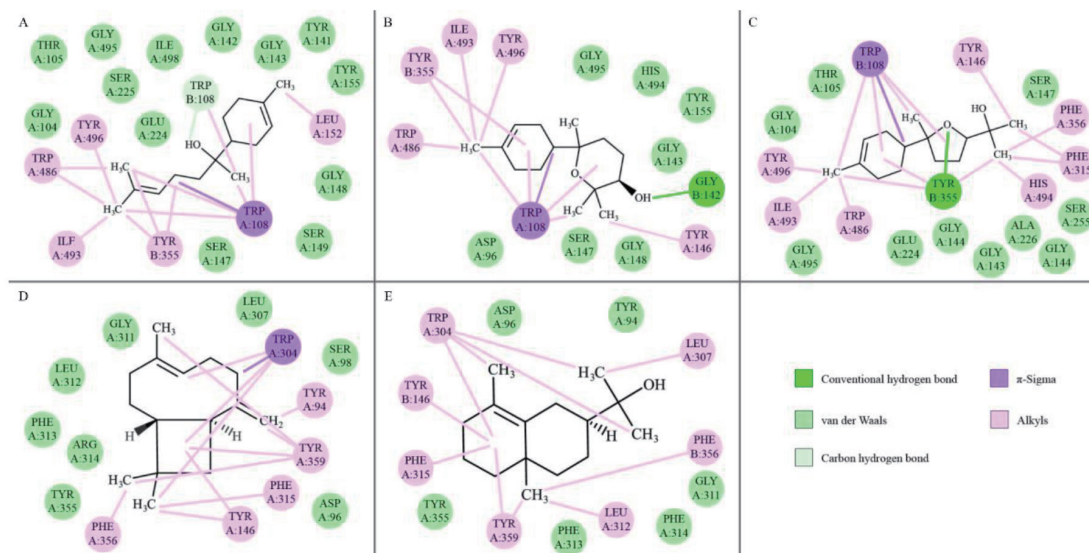


Fig. 3 Structures represent the interaction between the residues of the binding site and the A, B, C, D, and E represent the interactions between the residues of the binding site of *E. electricus* AChE and the ligands α -Bisabolol (A), α -Bisabolol Oxide A (B), α -Bisabolol Oxide B (C), β -Caryophyllene (D), and Epi- γ -Eudesmol (E).

idostigmine (-6.3 and -6.7 kcal.mol $^{-1}$) showed lower values when compared to some molecules under study (Tables 2 and 3).

3.2.2 ADME analysis shows low toxicity for the major molecules

Knowledge of the pharmacokinetic characteristics of absorption, distribution, metabolism, and excretion determines the potential performance of candidate drug molecules. It is believed that the pharmacokinetic properties of clinical trial candidates are critical parameters that potentially speed up trials. ADME parameters were used in order to estimate using *in silico* tools the ability of EOBU components to be possible candidates for developing new drugs. The Topological Polar Surface Area (TPSA) makes use of contributions from functional groups from analysis in databases of structures, where it measures the polar surface area of the molecule, avoiding the need for calculations from its three-dimensional (3D) structure, in addition to deciding which possible biological conformations are relevant for biological activity.

ADME analysis is a computational approach to drug design, assisting in the process of developing and discovering molecules of interest⁴¹. It is possible to reduce the time and cost of developing new drugs with pharmacological activity through *in silico* approaches. The screening of numerous compounds in short periods is facilitated by computational tools, since research laboratories without this support waste much time when performing the same activity. Additionally, computational results may be useful when determining the appropriate use and application of synthetic and semisynthetic drugs for a variety of purposes and diseases.

Most of the phytochemical compounds of EOBU did not show activities as an inhibitor/substrate of P-gp, except for Cubenol, Farnesol, Linalool, and Spathulene. P-gp is the main element of the ATP binding cassette transporter or ABC transporter. Its function is to protect the central nervous system (CNS) from xenobiotic activity. P-gp acts as an efflux transporter, decreasing the bioavailability of drugs and preventing their accumulation in the intracellular region⁴². Regarding gastrointestinal absorption (GI absorption), α -bisabolol, α -bisabolol Oxide A, and α -bisabolol Oxide B showed high absorption by the gastrointestinal system, while other molecules had low absorption. Some compounds showed permeability through the blood-brain barrier (BBB), except Valencene, α -Cadinene, α -Humulene, β -caryophyllene, β -Elemene, γ -Elemene, γ -Muurolene and δ -Cadinene (Table 3). According to⁴³, the Bioavailability Score measures the likelihood that a molecule will have bioavailability and permeability above 10% when administered to rats, needing at least a score ≥ 0.55 (all the oil components are equal to 0.55) to pass Lipinski's rule-of-five and, consequently, to be bioavailable and permeable in rats^{34, 43} (Fig. 4b). Synthetic accessibility assesses the likelihood of these compounds being produced artificially, ranging from 1 (easily assessed) to 10 (rarely assessed). Due to the fact that all the oil constituents are closer to 1 than to 10, they have a high probability of being synthetically produced³⁴. As stated by Egan *et al.* (2000)⁴⁴, effective absorption occurs when TPSA and WLogP are greater than 131.6 Å² and 5.688, respectively (Fig. 4a and Table 3). Thus, there were no violations of these rules found with any of the molecules examined here, regardless of whether they are FDA-approved or not⁴⁴.

Table 3 Swiss ADME results.

Molecule	MW ^a (g.Mol ⁻¹)	TPSA ^b (Å ²)	WLogP	BBB ^c Permeant	GI ^d Absorption	P-gp ^e Substrat	BAS ^f	SA ^g
Aromadendrene	204.35	0.00	4.27	Yes	Low	No	0.55	3.70
Camphene	136.23	0.00	3.00	Yes	Low	No	0.55	3.50
Caryophyllene Oxide	220.35	12.53	3.94	Yes	High	No	0.55	4.35
Copaene	204.35	0.00	4.27	Yes	Low	No	0.55	4.62
Cubenol	222.37	20.23	3.78	Yes	High	No	0.55	4.34
Epi-γ-Eudesmol	222.37	20.23	4.06	Yes	High	No	0.55	3.88
Eucalyptol	154.25	9.23	2.74	Yes	High	No	0.55	3.65
Farnesol	222.37	20.23	4.40	Yes	High	No	0.55	3.17
Linalool	154.25	20.23	2.67	Yes	High	No	0.55	2.74
Nerolidol	222.37	20.23	4.40	Yes	High	No	0.55	3.53
Spathulenol	220.35	20.23	3.39	Yes	High	No	0.55	3.78
Valencene	204.35	0.00	4.73	No	Low	No	0.55	4.22
α-Bisabolol	222.37	20.23	4.23	Yes	High	No	0.55	3.95
α-Bisabolol Oxide A	238.37	29.46	3.44	Yes	High	No	0.55	4.38
α-Bisabolol Oxide B	238.37	29.46	3.44	Yes	High	No	0.55	4.30
α-Cadinene	204.35	0.00	4.58	No	Low	No	0.55	4.35
α-Humulene	204.35	0.00	5.04	No	Low	No	0.55	3.66
α-Pinene	136.23	0.00	3.00	Yes	Low	No	0.55	4.44
α-Thujene	136.23	0.00	3.00	Yes	Low	No	0.55	3.99
β-Caryophyllene	204.35	0.00	4.73	No	Low	No	0.55	4.51
β-Elemene	204.35	0.00	4.75	No	Low	No	0.55	3.63
β-Pinene	136.23	0.00	3.00	Yes	Low	No	0.55	3.73
γ-Elemene	204.35	0.00	4.89	No	Low	No	0.55	3.81
γ-Murolene	204.35	0.00	4.58	No	Low	No	0.55	4.35
δ-Cadinene	204.35	0.00	4.73	No	Low	No	0.55	4.14
Donepezil ^h	379.49	38.77	3.83	Yes	High	Yes	0.55	3.62
Galanthamine ^h	287.45	41.93	1.32	Yes	High	Yes	0.55	4.57
Huperzine A ^h	223.29	29.54	1.94	Yes	High	Yes	0.55	2.26
Neostigmina ^h	275.35	44.81	1.01	Yes	High	Yes	0.55	3.38
Physostigmine ^h	181.21	33.42	0.57	Yes	High	No	0.55	2.21
Pyridostigmine ^h	250.34	32.78	2.44	Yes	High	No	0.55	2.73
Rivastigmine ^h	179.30	26.02	2.69	Yes	High	No	0.55	3.70
Tacrine ^h	198.26	38.91	2.70	Yes	High	Yes	0.55	2.08

^aMW: Molecular Weight; ^bTPSA: Topological Polar Surface Area; ^cBBB: Brain Blood Barrier; ^dGI: Gastrointestinal; ^eP-gp: P-glycoprotein; ^fBAS: Bioavailability Score; ^gSA: Synthetic Accessibility; ^hAcetylcholinesterase inhibitors drugs approved by the FDA.

3.2.3 Target prediction and enrichment analysis

Analyses of functional enrichment expand information on predicted molecular targets, resulting in detailed descriptions of possible interactions, the activity of natural and synthetic molecules at the level of biological functions,

as well as the biological processes in which they are involved.

GO enrichment annotations were classified into biological processes (BPs), cellular components (CCs), and molecular functions (MFs) (Fig. 5). Some genes were enriched

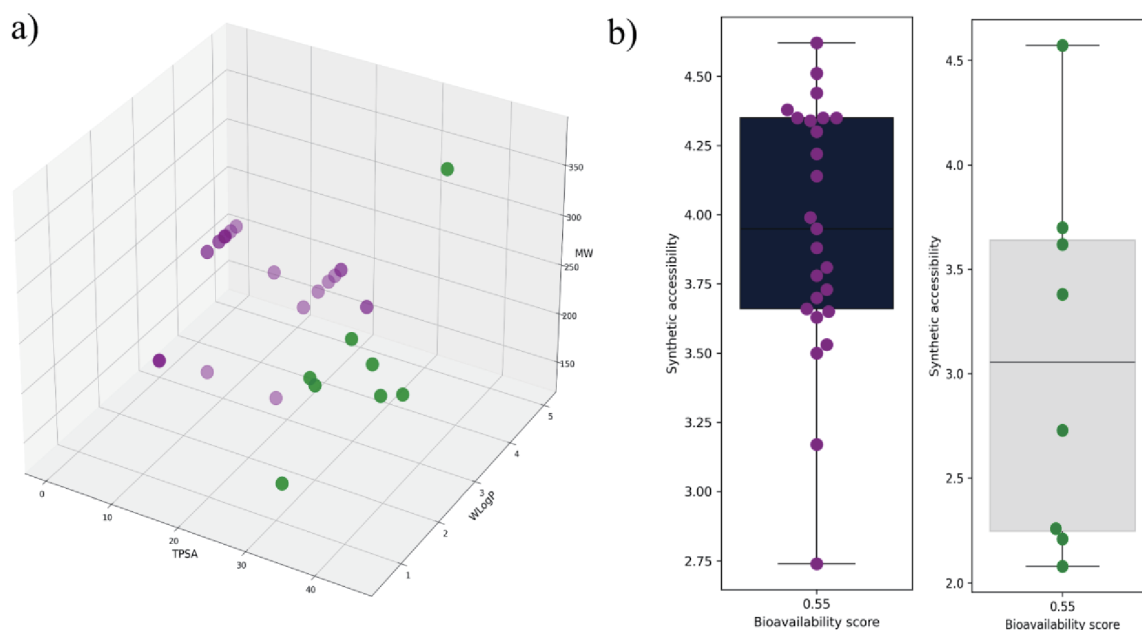


Fig. 4 3D Scatterplot graph representing the relationship between Topological Polar Surface Area (TPSA), lipophilicity (WLogP) and Molecular Weight (MW) of the 25 major molecules of the essential oil of *B. unguolata* (Purple) and FDA-approved drugs against AD (Green) (a) and Boxplot representation of Bioavailability Score (X axis) versus Synthetic Accessibility (Y axis) of the 25 major molecules (Purple) and FDA-approved (Green) (b).

in signaling pathways related to AD-amyloid secretase pathway, in addition to JAK/STAT, G-protein signaling pathway, and inflammation mediated by chemokine and cytokine signaling pathways. Most of the genes that make up the BPs were: Protein phosphorylation (14.3%), inflammatory response (12.8%), response to xenobiotic stimulus (9.6%), synaptic transmission (7.9%), positive regulation of ERK1 and ERK2 cascade (7.3%) and intracellular signal transduction (9.3%), while for MFs they were: ATP binding (20.7%), zinc ion binding (17.3%), identical protein binding (17.9%), protein serine/threonine kinase activity (11.2%) and enzyme binding (10.1%). As for the cellular location in which the gene products play a role, they presented the plasma membrane (52.3%), cytosol (43.4%), and cytoplasm (39.9%), suggesting the cell region where the gene products are active⁴⁵⁾.

There are profound relationships between BPs and AD notes, and some of these processes impact the development and progression of the disease. Abnormal protein phosphorylation is central to the dysfunction of molecular pathways in the aging brain. Consequently, abnormal protein phosphorylation has been linked to human diseases, including AD. The hyperphosphorylation of the TAU protein forms neurofibrillary tangles (NFTs) that are deposited in the brain tissue, in addition to the proteins MAP1B, dynein, and CRMP-2 that are also hyperphosphorylated in AD, evidencing the deregulation in the processes of phosphorylation of proteins^{46, 47)}. Thus, another study evaluated the potential of β -caryophyllene in phosphorylative pro-

cesses, observing the reduction in phosphorylation of MLKL proteins in rat brains, an important relationship since it constitutes mostly EOBUs and possibly has involvement in gene modulation in these processes⁴⁸⁾.

Several studies have evaluated inflammatory responses in the brain of individuals with AD, assuming that neuroinflammation may be the main neuropathological event causing neurodegeneration. Furthermore, high levels of cytokines have already been observed in the brains of AD patients⁴⁹⁾. The α -bisabolol is known for its activity in inflammatory disorders and the prevention of neurodegenerative diseases, in addition to causing a decrease in TNF- α in rats, with TNF- α being one of the markers of acute inflammation^{50, 51)}. The α -bisabolol may also contribute to gene modulation in response to inflammation, as evidenced in functional enrichment analyses.

Synaptic transmission is an essential biological process in the development and progression of AD. Loss of synapse is strongly related to the cognitive decline in AD since high concentrations of A β peptide interfere with synaptic neurotransmission in glutamatergic neurons^{52, 53)}.

3.3 Proton nuclear magnetic resonance (¹H NMR) of EOBUs

Previous data from our group has shown α -Bisabolol as the primary compound of EOBUs⁵⁴⁾. When analyzing the chemical shifts (δ) of EOBUs when in contact with AChE, the most substantial intensity peaks were observed in the spectrum when considering α -Bisabolol (Fig. 6). That evidence the significant changes in the chemical composition

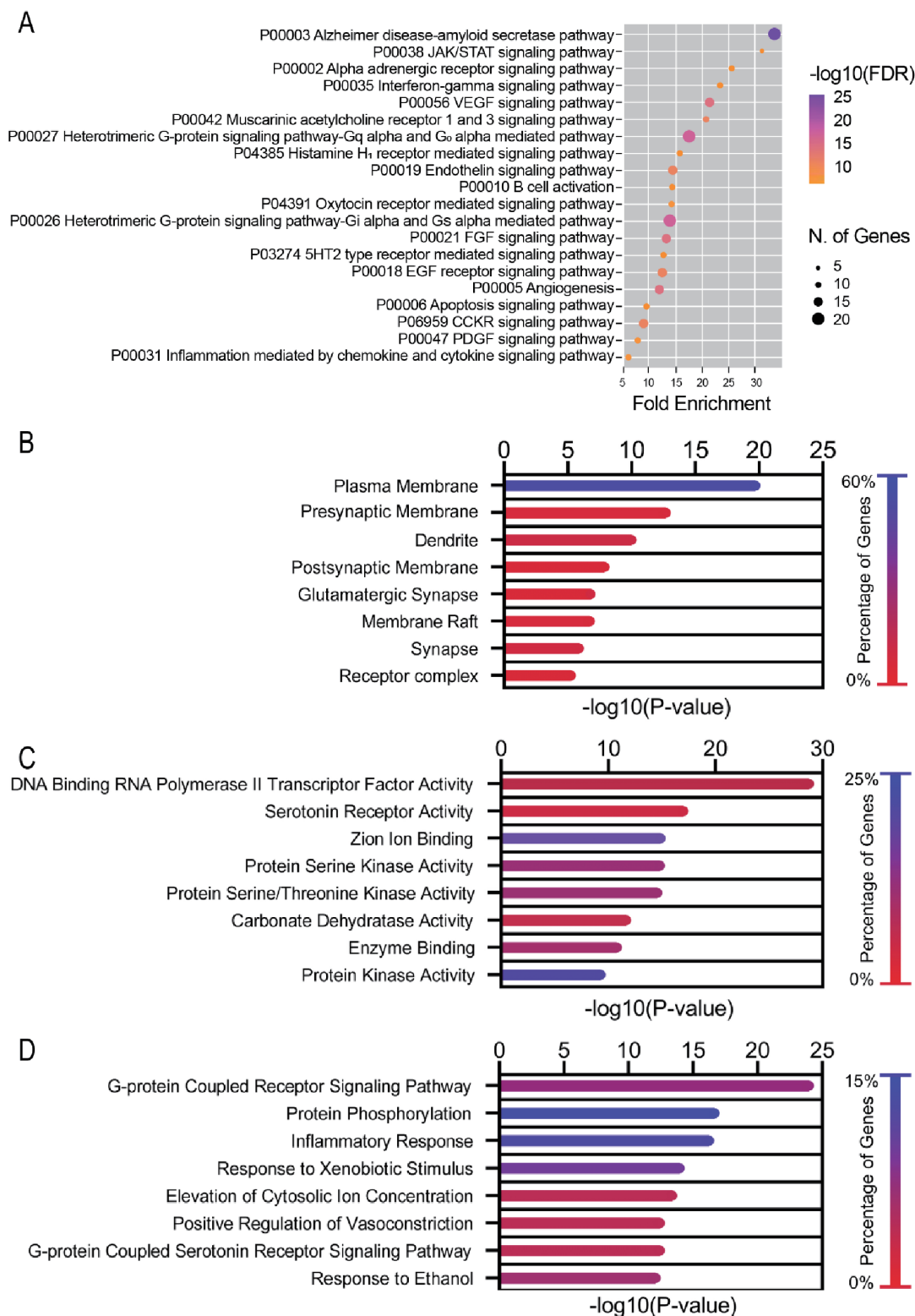


Fig. 5 ShinyGO KEGG Pathway enrichment analysis of the predicted targets for pathways, with a P-value cutoff(FDR) of 0.05. The fold enrichment is defined as the percentage of genes in your list belonging to a pathway(a)and FunRich GO enrichment analysis of the predicted targets for Cellular component, Molecular function and Biological process, respectively (b-d).

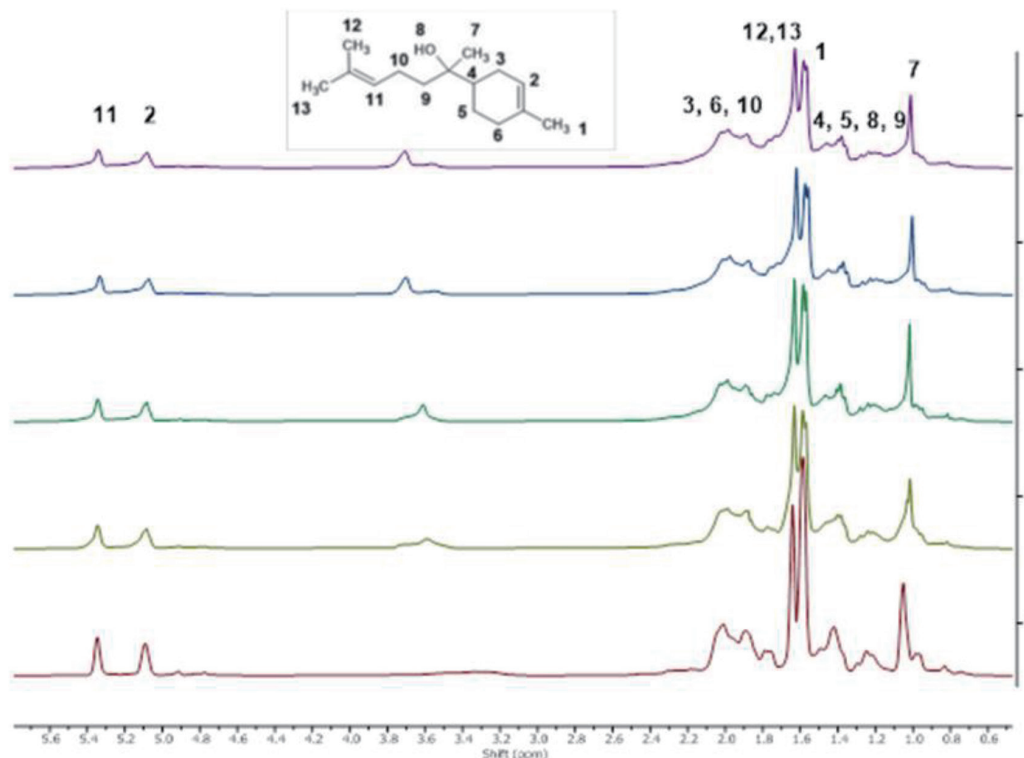


Fig. 6 EOBU ^1H NMR Spectrum. ^1H NMR titration of EOBU essential oil with AChE (0.67 mg.mL^{-1}) in DMSO. Titration was performed with AChE at $0 \text{ }\mu\text{L}$ (red), $5 \text{ }\mu\text{L}$ (light green), $10 \text{ }\mu\text{L}$ (dark green), $15 \text{ }\mu\text{L}$ (blue) and $20 \text{ }\mu\text{L}$ (purple). Above the purple line is the structure of α -Bisabolol.

Table 4 Chemical changes of protons caused after interactions of α -Bisabolol with acetylcholinesterase.

Assignment	Shift δ (ppm) 0 μL	Shift δ (ppm) 5 μL	Shift δ (ppm) 10 μL	Shift δ (ppm) 15 μL	Shift δ (ppm) 20 μL	shift $\Delta\delta$ (ppm) 0-20 μL
1	1.6403	1.6326	1.6320	1.6285	1.6212	0.0191
7	1.0597	1.0154	1.0181	1.0127	1.0039	0.0558
12, 13	1.5864	1.5762	1.5753	1.5718	1.5659	0.0205

δ (ppm) = chemical shift, $\Delta\delta$ (ppm) = variation of chemical shifts of the protons 1,7,12 and 13 of the major compounds of EOBU α -Bisabolol after titration with AChE at concentrations from 0 to 20 μL .

of the environment where hydrogens n $^{\circ}$ 1, 7, 12 and 13 are found (Table 4). In all cases, there was a shift to the high field (upfield), showing the presence of electron-donating groups, whose effects increased the electron density around the analyzed hydrogens due to AChE-EOBU interactions. α -Bisabolol possibly interacts with the AChE enzyme, corroborating the H changes analyzed in the chemical shifts (δ) of the essential oil. When in contact with AChE Comparing the H present in α -Bisabolol, a greater difference in the chemical shift ($\Delta\delta$) in hydrogen 7 is noted. This finding suggests a greater interaction between this hydrogen and AChE. Based on *in silico* analysis, α -bisabolol also interacts with all hydrogen atoms noted with a chemical shift in the ^1H NMR spectrum, supporting the accuracy of this finding (Fig. 3a), as this compound showed excellent binding affinity. with the active site of

this enzyme.

4 Conclusions

The development of new drugs in this line of research is extremely important for elucidating the factors that cause Alzheimer's disease, as well as obtaining an effective treatment or cure for this disease. Our studies carried out in the Amazon Region in Roraima, showed that α -Bisabolol, the major component present in the essential oil of *Bauhinia unguolata* (EOBU), can become a strong candidate for a drug as an acetylcholinesterase inhibitor and can be used in the treatment of Alzheimer's disease. In this sense, continuous studies are necessary. Due to the extensive structural diversity of the compounds identified in the EOBU,

we can anticipate that new discoveries can and have yet to be made as a result. EOBU should be screened for its neural enhancement effects. The interactions between α -Bisabolol and the main essential oil compounds with the AChE receptor were significant, as well as the ADME parameters, where data on its pharmacokinetics can contribute to a better physiological understanding of the use of EOBU. Whether enhanced integration of EOBU into the regular diet or ingestion of supplements can stabilize AChE function remains to be explored.

Author Contributions Statement

In this study, S.R.N.A.M. designed and conducted the research, performed data curation, and contributed to the writing and review of the original manuscript. A.J.S. and L.A.L.P. conducted molecular docking and enrichment analysis and assisted in manuscript editing. T.M.M. contributed to ¹H NMR analysis. A.C.G.R.M. and I.C.B. aided in data curation, formal analysis, and reviewed and edited the original abstract. P.G. defined resources, supervised, and validated results, and assisted in abstract review and editing. R.M.G.M. assisted in manuscript writing and result validation. J.L.N. and A.A.M.F. managed project administration, supervision, conceptualization, data curation, funding acquisition, and original article writing.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess23148

References

- 1) Dugger, B.N.; Dickson, D.W. Pathology of neurodegenerative diseases. *Cold Spring Harb. Perspect. Biol.* **9**, a028035 (2017). doi: 10.1101/cshperspect.a028035
- 2) Vidal, C.; Zhang, L. An analysis of the neurological and molecular alterations underlying the pathogenesis of Alzheimer's disease. *Cells* **10**, 546 (2021). doi: 10.3390/cells10030546
- 3) Atri, A. The Alzheimer's disease clinical spectrum: Diagnosis and management. *Med. Clin. North Am.* **103**, 263-293 (2019). doi: 10.1016/j.mcna.2018.10.009
- 4) Wimo, A.; Seeher, K.; Cataldi, R.; Cyhlarova, E.; Dieleman, J.L. *et al.* The worldwide costs of dementia in 2019. *Alzheimer's Dement.* **19**, 2865-2873 (2023). doi: 10.1002/alz.12901
- 5) Trushina, E.; Mielke, M.M. Recent advances in the application of metabolomics to Alzheimer's disease. *Biochim. Biophys. Acta - Mol. Dis.* **1842**, 1232-1239 (2014). doi: 10.1016/j.bbadis.2013.06.014
- 6) Passeri, E.; Elkhoury, K.; Morsink, M.; Broersen, K.; Linder, M. *et al.* Alzheimer's disease: Treatment strategies and their limitations. *Int. J. Mol. Sci.* **23**, 13954 (2022). doi: 10.3390/ijms232213954
- 7) Marucci, G.; Buccioni, M.; Ben, D.D.; Lambertucci, C.; Volpini, R. *et al.* Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology* **190**, 108352 (2020). doi: 10.1016/j.neuropharm.2020.108352
- 8) Khoury, R.; Rajamanickam, J.; Grossberg, G.T. An update on the safety of current therapies for Alzheimer's disease: Focus on rivastigmine. *Ther. Adv. Drug Saf.* **9**, 171-178 (2018). doi: 10.1177/2042098617750555
- 9) Ali, T.B.; Schleret, T.R.; Reilly, B.M.; Chen, W.Y.; Abagyan, R. Adverse effects of cholinesterase inhibitors in dementia, according to the pharmacovigilance databases of the United-States and Canada. *PloS One* **10**, e0144337 (2015). doi: 10.1371/journal.pone.0144337
- 10) Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils - A review. *Food Chem. Toxicol.* **46**, 446-475 (2008). doi: 10.1016/j.fct.2007.09.106
- 11) Deyno, S.; Mtewa, A.G.; Abebe, A.; Hymete, A.; Makonnen, E. *et al.* Essential oils as topical anti-infective agents: A systematic review and meta-analysis. *Complement. Ther. Med.* **47**, 102224 (2019). doi: 10.1016/j.ctim.2019.102224
- 12) Valdivieso, M.U.; Gomez, C.L.; Plaza, J.D.; Gil, A. Antimicrobial, antioxidant, and immunomodulatory prop-

- erties of essential oils: A systematic review. *Nutrients* **11**, 2786 (2019). doi: 10.3390/nu11112786
- 13) Miguel, M.G. Antioxidant and anti-inflammatory activities of essential oils: A short review. *Molecules* **15**, 9252-9287 (2010). doi: 10.3390/molecules15129252
- 14) Friedli, M.J.; Inestrosa, N.C. Huperzine A and its neuroprotective molecular signaling in Alzheimer's disease. *Molecules* **26**, 6531 (2021). doi: 10.3390/molecules26216531
- 15) Tang, J.J.; Huang, L.F.; Deng, J.L.; Wang, Y.M.; Guo, C. *et al.* Cognitive enhancement and neuroprotective effects of OABL, a sesquiterpene lactone in 5xFAD Alzheimer's disease mice model. *Redox Biol.* **50**, 102229 (2022). doi: 10.1016/j.redox.2022.102229
- 16) Martins, R.M.; Xavier, F.H.J.; Barros, M.R.; Menezes, T.M.; de Assis, C.R. *et al.* Impact on cholinesterase-inhibition and *in silico* investigations of sesquiterpenoids from Amazonian *Siparuna guianensis* Aubl. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **252**, 119511 (2021). doi: 10.1016/j.saa.2021.119511
- 17) Fortunato, R.H.; Nores, M.J. "Cow's hoof" (*Bauhinia* L., Leguminosae): A review on pharmacological properties of Austral South American species. *Plants* **12**, 31-31 (2022). doi: 10.3390/plants12010031
- 18) Filho, V.C. Chemical composition and biological potential of plants from the genus *Bauhinia*. *Phytother. Res.* **23**, 1347-1354 (2009). doi: 10.1002/ptr.2756
- 19) de Sousa, L.M.; de Carvalho, J.L.; da Silva, H.C.; Lemos, T.L.; Arriaga, A.M. *et al.* New cytotoxic bibenzyl and other constituents from *Bauhinia unguolata* L. (Fabaceae). *Chem. Biodivers.* **13**, 1630-1635 (2016). doi: 10.1002/cbdv.201600058
- 20) de Souza, A.J.B.; Ocampos, F.M.M.; Pulgrossi, R.C.; Dokkedal, A.L.; Colnago, L.A. *et al.* NMR-based metabolomics reveals effects of water stress in the primary and specialized metabolisms of *Bauhinia unguolata* L. (Fabaceae). *Metabolites* **13**, 381 (2023). doi: 10.3390/metabo13030381
- 21) Kaurinovic, B.; Vastag, D. Flavonoids and phenolic acids as potential natural antioxidants. in *Antioxidants* (Shalaby, E. ed.), *IntechOpen* [S.l.: s.n.] (2019). doi: 10.5772/intechopen.83731
- 22) dos Santos, K.M.; Gonçalves, P.S.; de Paiva, M.J.N.; Lacerda, G.A. Acetylcholinesterase inhibition starting from extracts of *Bauhinia variegata* L., *Bauhinia* var. *candida* (Aiton) Buch.-Ham., and *Bauhinia unguolata* L. *Rev. Soc. Bras. Med. Trop.* **44**, 781-783 (2011). doi: 10.1590/s0037-86822011000600025
- 23) Medeiros, S.R.N.A.; Filho, A.A.F.; Costa, H.N.R.; dos Silva, F.S.; dos Santos, R.C. *et al.* Chemical profile, antimicrobial activity, toxicity on *Artemia salina* and anti-acetylcholinesterase enzyme essential oil from *Bauhinia unguolata* L. (Fabaceae) leaves. *J. Med. Plants Res.* **10**, 442-449 (2016). doi: 10.5897/jmpr.2016.61205
- 24) Silva, A.M.A.; Silva, H.C.; Monteiro, A.O.; Lemos, T.L.G.; de Souza, S.M. *et al.* Chemical composition, larvicidal and cytotoxic activities of the leaf essential oil of *Bauhinia cheilantha* (Bong.) Steud. *S. Afr. J. Bot.* **131**, 369-373 (2020). doi: 10.1016/j.sajb.2020.03.011
- 25) Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254 (1976). doi: 10.1016/0003-2697(76)90527-3
- 26) Sedmak, J.J.; Grossberg, S.E. A rapid, sensitive, and versatile assay for protein using coomassie brilliant blue G250. *Anal. Biochem.* **79**, 544-552 (1977). doi: 10.1016/0003-2697(77)90428-6
- 27) Kim, S.; Chen, J.; Cheng, T.; Gindulyte, A.; He, J. *et al.* PubChem 2023 update. *Nucleic Acids Res.* **51**, D1373-D1380 (2022). doi: 10.1093/nar/gkac956
- 28) Cheung, J.; Rudolph, M.J.; Burshteyn, F.; Cassidy, M.S.; Gary, E.N. *et al.* Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *J. Med. Chem.* **55**, 10282-10286 (2012). doi: 10.1021/jm300871x
- 29) Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G. *et al.* Swiss-model: Homology modelling of protein structures and complexes. *Nucleic Acids Res.* **46**, 296-303 (2018). doi: 10.1093/nar/gky427
- 30) Nachon, F.; Carletti, E.; Ronco, C.; Trovaslet, M.; Nicolet, Y. *et al.* Crystal structures of human cholinesterases in complex with huprine W and tacrine: Elements of specificity for anti-Alzheimer's drugs targeting acetyl- and butyryl-cholinesterase. *Biochem. J.* **453**, 393-399 (2013). doi: 10.1042/BJ20130013
- 31) Santana, C.A.; Silveira, S.D.A.; Moraes, J.P.; Izidoro, S.C.; Minardi, R.C.M. *et al.* GRASP: A graph-based residue neighborhood strategy to predict binding sites. *Bioinformatics* **36**, 726-734 (2020). doi: 10.1093/bioinformatics/btaa805
- 32) Furqan, T.; Batool, S.; Habib, R.; Shah, M.; Kalasz, H. *et al.* Cannabis constituents and acetylcholinesterase interaction: Molecular docking, *in vitro* studies and association with CNR1 rs806368 and AChE rs17228602. *Biomolecules* **10**, 758 (2020). doi: 10.3390/biom10050758
- 33) Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **31**, 455-461 (2009). doi: 10.1002/jcc.21334
- 34) Daina, A.; Michielin, O.; Zoete, V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* **7**, 42717 (2017). doi: 10.1038/srep42717

- 35) Daina, A.; Michielin, O.; Zoete, V. SwissTargetPrediction: Updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* **47**, 357-364 (2019). doi: 10.1093/nar/gkz382
- 36) Pathan, M.; Keerthikumar, S.; Ang, C.S.; Gangoda, L.; Quek, C.Y. *et al.* FunRich: An open access standalone functional enrichment and interaction network analysis tool. *Proteomics* **15**, 2597-2601 (2015). doi: 10.1002/pmic.201400515
- 37) Ge, S.X.; Jung, D.; Yao, R. ShinyGO: A graphical gene-set enrichment tool for animals and plants. *Bioinformatics* **36**, 2628-2629 (2019). doi: 10.1093/bioinformatics/btz931
- 38) Mota, W.M.; Barros, M.L.; Cunha, P.E.L.; Santana, M.V.A.; Stevam, C.S. *et al.* Avaliação da inibição da acetilcolinesterase por extratos de plantas medicinais. *Rev. Bras. Plantas Med.* **14**, 624-628 (2012). doi: 10.1590/s1516-05722012000400008
- 39) Shanmuganathan, B.; Suryanarayanan, V.; Sathya, S.; Narenkumar, M.; Singh, S.K. *et al.* Anti-amyloidogenic and anti-apoptotic effect of α -bisabolol against A β induced neurotoxicity in PC12 cells. *Eur. J. Med. Chem.* **143**, 1196-1207 (2018). doi: 10.1016/j.ejmech.2017.10.017
- 40) Eddin, L.B.; Jha, N.K.; Goyal, S.N.; Agrawal, Y.O.; Subramanya, S.B. *et al.* Health benefits, pharmacological effects, molecular mechanisms, and therapeutic potential of α -bisabolol. *Nutrients* **14**, 1370 (2022). doi: 10.3390/nu14071370
- 41) Wu, F.; Zhou, Y.; Li, L.; Shen, X.; Chen, G. *et al.* Computational approaches in preclinical studies on drug discovery and development. *Front. Chem.* **8**, 760 (2020). doi: 10.3389/fchem.2020.00726
- 42) Srivalli, K.M.R.; Lakshmi, P.K. Overview of P-glycoprotein inhibitors: A rational outlook. *Braz. J. Pharm. Sci.* **48**, 353-367 (2012). doi: 10.1590/s1984-82502012000300002
- 43) Martin, Y.C. A bioavailability score. *J. Med. Chem.* **48**, 3164-3170 (2005). doi: 10.1021/jm0492002
- 44) Egan, W.J.; Merz, K.M.; Baldwin, J.J. Prediction of drug absorption using multivariate statistics. *J. Med. Chem.* **43**, 3867-3877 (2000). doi: 10.1021/jm000292e
- 45) Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H. *et al.* Gene ontology: Tool for the unification of biology. *Nat. Genet.* **25**, 25-29 (2000). doi: 10.1038/75556
- 46) Henrique, A.G.; Müller, T.; Oliveira, J.M.; Cova, M.; Silva, C.B.C.; Silva, O.A.B.C. Altered protein phosphorylation as a resource for potential AD biomarkers. *Sci. Rep.* **6**, 30319 (2016). doi: 10.1038/srep30319
- 47) Ferrer, I.; Benito, P.A.; Ausín, K.; Pamplona, R.; Del Rio, J.A. *et al.* Dysregulated protein phosphorylation: A determining condition in the continuum of brain aging and Alzheimer's disease. *Brain Pathol.* **31**, 12996 (2021). doi: 10.1111/bpa.12996
- 48) Yang, M.; Lv, Y.; Tian, X.; Lou, J.; An, R. *et al.* Neuroprotective effect of β -caryophyllene on cerebral ischemia-reperfusion injury via regulation of necroptotic neuronal death and inflammation: *In vivo* and *in vitro*. *Front. Neurosci.* **11**, 583 (2017). doi: 10.3389/fnins.2017.00583
- 49) Uddin, M.S.; Kabir, M.T.; Jalouli, M.; Rahman, M.A.; Jeandet, P. *et al.* Neuroinflammatory signaling in the pathogenesis of Alzheimer's disease. *Curr. Neuropharmacol.* **20**, 126-146 (2022). doi: 10.2174/1570159x19666210826130210
- 50) Rocha, N.F.M.; Rios, E.R.V.; Carvalho, A.M.R.; Cerqueira, G.S.; Lopes, A.D.A. *et al.* Anti-nociceptive and anti-inflammatory activities of (-)- α -bisabolol in rodents. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **384**, 525-533 (2011). doi: 10.1007/s00210-011-0679-x
- 51) Ramazani, E.; Akaberi, M.; Emami, S.A. Tayarani-Najaran Z. Pharmacological and biological effects of alpha-bisabolol: An updated review of the molecular mechanisms. *Life Sci.* **304**, 120728 (2022). doi: 10.1016/j.lfs.2022.120728
- 52) Alifragis, P.; Marsh, J. Synaptic dysfunction in Alzheimer's disease: The effects of amyloid beta on synaptic vesicle dynamics as a novel target for therapeutic intervention. *Neural Regen. Res.* **13**, 616 (2018). doi: 10.4103/1673-5374.230276
- 53) Subramanian, J.; Savage, J.C.; Tremblay, M.È. Synaptic loss in Alzheimer's disease: Mechanistic insights provided by two-photon *in vivo* imaging of transgenic mouse models. *Front. Cell. Neurosci.* **14**, 592607 (2020). doi: 10.3389/fncel.2020.592607
- 54) Medeiros, S.R.N.A.; Bezerra, I.C.; Silva, A.J.; Pedroza, L.A.L.; Martins, R.M.G. *et al.* *In vitro* and *in silico* antioxidant activity and molecular characterization of *Bauhinia unguolata* L. essential oil. *Chem. Biodivers.* **21**, 1-8 (2024). doi: 10.1002/cbdv.202301536

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