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POTENTIAL OF THE ESSENTIAL OIL OF ALPINIA ZERUMBET (PERS.) B.L. BURTT & R. M. SM. (COLONIA) AGAINST ALZHEIMER'S DISEASE, THROUGH ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITY IN VITRO AND IN SILICO STUDIES

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ABSTRACT

The plant Alpinia zerumbet, commonly known as "Colonia," is widely utilized in traditional medicine for addressing anxiety and hypertension, both of which are associated with oxidative damage in the brain. This study aimed to explore the antioxidant and anticholinesterase properties of the essential oil extracted from its leaves. Additionally, an in-silico investigation was carried out on several essential oil constituents concerning the acetylcholinesterase enzyme. The GC/MS analysis identified nineteen volatile compounds, mainly monoterpenoids (98.41%), particularly terpinen-4-ol, eucalyptol, γ-terpinene, and sabinene, standing out as the major components. The anticholinesterase and radical-scavenging activities exhibited by A. zerumbet essential oil proved to be noteworthy when compared to standard drugs. In the molecular docking analysis, the compounds α-terpinene, β-pinene, eucalyptol, and terpinolene were found to bind to the same region as the standard galantamine binding site, suggesting their similar action. Conversely, o-cymene bound to a distinct region from the galantamine binding site, hinting at a potential synergistic effect with the co-crystallized inhibitor on AChE. Therefore, A. zerumbet essential oil, given its established pharmacological properties in the central nervous system (CNS) and the presence of antioxidant substances and acetylcholinesterase inhibitors, emerges as a promising candidate for more targeted pharmacological investigations related to Alzheimer's disease.

Keywords: Cholinergic Hypothesis. Free Radicals. Molecular Docking.

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INTRODUCTION

The plant *Alpinia zerumbet* (Pers.) Burtt. et Smith belongs to the Zingiberaceae family and is originated from Asia but has gained prominence in various parts of South America as an ornamental plant. In Northeastern Brazil, it is popularly known as "Colonia" and is often used in traditional medicine to treat conditions such as hypertension and anxiety (Da Cunha et al., 2013). Studies show that the plant's antihypertensive and anxiolytic properties can be attributed in part to the presence of flavonoids, such as (+)-catechin, (-)-epicatechin and alpinetin (Costa et al. 1998).

The flavonoids identified in the aqueous extract of *A. zerumbet* as rutin, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucuronide, catechin and epicatechin are well known substances that contribute to the hypotensive, diuretic and antiulcerogenic activity of the plant and the compounds dihydro-5,6-dehydrokavaine and 5,6-dehydrokavaine act as antiulcerogenic and antithrombotic agents (Mpalantinos et al. 1998).

The studies by Zoghbi et al. (1999) identified the main components of the essential oil of the leaves and flowers of *A. zerumbet*, among which terpinen-4-ol, present in both leaves and flowers, has an antihypertensive action. In addition to terpinen-4-ol, which accounts for 22.7% of the compounds, the leaves also contain limonene (25.1%) and γ-terpinene (17.4%), while the flowers contain 1.8-cineol (23.1%) and sabinene (14.5%).

Terpinen-4-ol is the main constituent of *A. zerumbet* essential oil. The psychopharmacological and electrophysiological activities of this compound was investigated in male Swiss mice and Wistar rats. The compound was administered intraperitoneally and intracerebroventricularly. *For in* vitro experiments, terpinen-4-ol inhibited pentylenetetrazol (PTZ) induced seizures, indicating anticonvulsant effects. Electroencephalographic recordings showed that terpinen-4-ol protected against PTZ-induced seizures, corroborating the behavioral results. Terpinen-4-ol exerts anticonvulsant effects via regulation of GABAergic neurotransmission (Nóbrega et al. 2014).

Anxiety is more common in patients with hypertension, and these two conditions frequently coexist. More emphasis has been placed on determining etiology in patients with comorbid hypertension and anxiety. Increased interleukin IL-6, IL-17, and ROS accelerate the development of hypertension and anxiety (Qiu et al, 2023).

Acetylcholine has a crucial role in the peripheral and central nervous systems. Cholinergic neurons located in the basal forebrain, including the neurons that form the nucleus basalis of Meynert, are severely lost in Alzheimer's disease (AD). Synaptic loss is

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the principal correlate of disease progression and loss of cholinergic neurons contributes to memory and attention deficits. Thus, drugs that act on the cholinergic system represent a promising option to treat AD patients (Ferreira-Vieira et al. 2016).

Alzheimer's disease (AD) is a neurodegenerative disease caused by the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles. Acetylcholinesterase (AChE) is the crucial enzyme in the hydrolysis of one of the most extensively known neurotransmitters acetylcholine (ACh) that has been associated with the pathophysiology of AD. Then, enzymatic inhibition of AChE activity has been an interesting treatment strategy for AD. Several natural products have been investigated worldwide aiming to discover new anticholinesterase agents that could be used as a therapeutic option for AD treatment (Taqui et al. 2022)

Acetylcholinesterase (AChE) inhibitors are an important therapeutic strategy in Alzheimer's disease, and efforts are being made in search of new molecules with anti-AChE activity. Most of the drugs currently available for the treatment of AD are AChE inhibitors: tacrine, donepezil, rivastigmine and galantamine, all of which have limited effectiveness and present some kind of side effects. The two last compounds are obtained from plants, which are potential sources of new inhibitors of AChE. These inhibitors according to the cholinergic hypothesis, increases the levels of the neurotransmitter acetylcholine in the brain, thus improving cholinergic functions in patients with Alzheimer's disease and alleviating the symptoms of this neurological disorder (Murray et al. 2013).

This plant shows potential in both cardiovascular and mental health issues, and it is therefore of interest in the context of Alzheimer's disease (AD). This neurodegenerative condition mainly affects the elderly population, highlighting the importance AChE inhibitors as a therapeutic target. In the case of *A. zerumbet*, the study seeks to explore its antioxidant and anticholinesterase activities, positioning it as a possible source of bioactive principles in the treatment of AD (Volpe et al. 2018).

Free radicals, characterized by their high reactivity, have the potential to influence a variety of cellular processes, both physiological and pathophysiological in organisms. High concentrations of these free radicals can trigger various neurodegenerative diseases, including Alzheimer's and Parkinson's (Bugger et al. 2020; Zhu et al. 2023).

Thus, *Alpinia zerumbet* emerges not only as an ornamental plant, but also as a valuable resource, providing a synergy between its bioactive flavonoid constituents, pharmacological properties and potential health benefits, especially in the approach to

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Alzheimer's disease, in which the chemical composition and its antioxidant and anticholinesterase activities will be *in vitro* and *in silico* evaluated.

EXPERIMENTAL

MATERIAL COLLECTION AND PROCESSING

The plant was collected in the garden of the Brazilian Agricultural Research Corporation (Embrapa Agroindústria Tropical), located in Bairro do Pici, in the city of Fortaleza, in the state of Ceará, in April 2022 with the coordinates lat: -3.7527757, log: -38.5756348. The exsiccate of the plant was deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará (UFC) under the code (EAC 65331). The essential oil was extracted using about 600 g of fresh leaves, using the hydrodistillation technique in a Clevenger-type apparatus.

GAS CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY (GC/MS)

The oil analysis was carried out on the Shimadzu QP-2010 equipment, under the following conditions: Rxt-5MS chromatographic column (Crossbond 5%, diphenyl/95% dimethylpolysiloxane), capillary (30m x 0.25mm x 0.25 µm) coated with fused silica; Helium as the carrier gas (24.2 mL/min), at a constant linear velocity; injector temperature at 250ºC (split mode 1:100); detector temperature at 250ºC. Additionally, the heating ramp was programmed, initially from 35ºC to 180ºC, with an increase of 4ºC/min up to 180ºC and then 17ºC/min up to 280ºC, remaining at this temperature for the final 10 minutes. Thus, the chromatogram was generated, which relates the retention time to the sample peaks. The mass spectrum was obtained by electron impact with a beam energy of 70 eV. In this way, mass spectra were generated, and the equipment suggests some compounds through a comparison with an existing NIST library. To effectively identify the oil components, mass spectra and retention indices (KI) were compared with those from literature - according to the NIST and Adams (2017) databases. The calculation of the experimental Kovat Index was performed by linear regression with interpolation of main compounds KI and their respective retention times of chromatogram.

IN VITRO EVALUATION OF ACETYLCHOLINESTERASE INHIBITION

The anticholinesterase activity (AChE) was measured in 96-well flat-bottom plates using the Elisa BIOTEK reader, model ELX 800, software "Gen5 V2.04.11" (ELLMAN et al.,

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1961). In the 96-well plates, the following solutions were used per well: 25 µL of acetylthiocholine iodide (15 mM), 125 µL of 5,5'-dithiobis-[2-nitrobenzoic acid] in Tris/HCL solution (50 nM, pH=8, with 0.1 M NaCl and 0.02 M $MgCL_2.6H_2O$ (3 mM, DTNB or Ellman's reagent)), 50 µL of Tris/HCL solution (50 nM, pH=8, with 0.1% bovine serum albumin (BSA)), 25 µL of the oil sample dissolved in methanol and diluted 10 times in Tris/HCL solution (50 mM, pH=8) to obtain a final concentration of 0.2 mg mL^{-1} (Rhee et al., 2001) (Trevisan et al., 2003). The dilutions of the samples and positive standards used in the quantitative evaluations in the microplate started from a stock solution with a concentration of 2 mg mL^{-1}, and were: 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg mL^{-1} Values related to the natural colorations of the oil were excluded from the analysis.

ANTIOXIDANT ACTIVITY BY DPPH METHOD

The antioxidant potential was measured in 96-well flat-bottom plates using an Elisa BioTek reader, model ELX 800, software "Gen5 V2.04.11" (Becker et al. 2019) with some modifications. In the 96-well plates, the following solutions were used per well: 180 µL of methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), 20 µL of the essential oil sample dissolved in methanol and diluted 10 times to obtain a final concentration result of 0.2 mg mL^{-1}. The oil concentrations were produced using the initial concentration solution of 2 mg mL^{-1}, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg mL^{-1}. The absorbances were read at 490 nm for a total of 60 minutes of incubation. The standard used for comparison was BHT. All samples were analyzed in triplicate.

ANTIOXIDANT ACTIVITY BY ABTS+• METHOD

The ABTS+• solution (7 mM, 5 ml) was mixed with 88 µL of potassium persulfate (140 mM). The mixture was stirred and stored in the dark at room temperature for 16 hours. Subsequently, 1 mL of this solution was added to 99 mL of ethanol. The absorbance is read at 734 nm. Several solutions of decreasing concentrations of essential oil from A. zerumbet were prepared, and 3.0 ml of ABTS+• solution was added to 30 µl of these solutions after 6 minutes; readings were taken at 734 nm (Re et al. 1999). The IC_{50} (half-maximal inhibitory concentration) was calculated by linear regression.

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MOLECULAR DOCKING

Preparation of ligand and receptor for docking simulations

The chemical structure of the compounds α-terpinene, α-thujene, β-pinene, limonene, 1,8-cineole, γ-terpinene, *o*-cymene, sabinene, terpinolene, and terpinen-4-ol were drawn using the MarvinSketch code. The lowest energy conformers were saved and subsequently optimized using the Avogadro code (Hanwell et al. 2012), configured to use the steepest descent algorithm with 50 iterations cycles, applying the MMFF94 (Merck Molecular Force Field) force field (HALGREN, 1996) (Silva et al. 2020).

The mechanism of action of the compounds against acetylcholinesterase (AChE) was evaluated *in silico* through molecular docking simulations, where the target structure was obtained from the Protein Data Bank (https://www.rcsb.org/), PDB ID: 4EY6 (CHEUNG et al., 2012). In the target preparation step, the residues were removed, polar hydrogens were added, and Gasteiger charges were calculated (Yan et al. 2014) using the Autodocktools ™ code (Huey et al. 2012).

Molecular docking simulations and data output

Fifty molecular docking simulations were performed against AChE with grid parameters centered to encompass the entire enzyme structure using the following axes: -0.412 (x), -51.388 (y), and 4.1 (z), with size parameters of 84Å (x), 92Å (y), and 126Å (z) using the AutoDockVina code (Trott; Olson 2010), Lamarkian Genetic Algorithm (LGA), and an exhaustiveness of 64 (Marinho et al., 2020). The statistical parameter RMSD (Root Mean Square Deviation) with values up to 2.0 Å (Yusuf et al. 2008) and a boundary affinity energy of -6.0 kcal/mol (Shityakov; Förster, 2014) were used to select the best pose for each ligand against AChE.

METHOD VALIDATION

The inhibitor Galantamine (GNT), co-crystallized in the AChE target (Cheung et al. 2012), was subjected to the redocking technique to validate the molecular docking simulations performed. The generated complex was compared to the native complex through structure superposition to confirm the reliability of the molecular docking and the software used as a tool to accurately simulate receptor-ligand interactions (Antopoulou et al. 2022).

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VISUALIZATION OF BINDING MODES AND ACHE-LIGAND INTERACTIONS

The binding modes and AChE-ligand interactions were visualized using UCSF Chimera™ (Pettersen et al. 2004). The complexes were saved in PDB format using PyMOL (Delano 2004). The reported molecular interactions and hydrogen bonds were calculated using the Discovery Studio Visualizer™ viewer (Biovia 2016).

RESULTS AND DISCUSSION

The composition of the leaf essential oil of *A. zerumbet* from Rio de Janeiro (Southeast Brazil) showed variations in content and composition between April and August 2005. In April, the main constituents identified in oil of *A. zerumbet* were terpinen-7-al (40.5%) and sabinene hydrate (15.4%); in August, the major components identified were terpinen-4-ol (29.4%) and 1,8-cineole (23.1%) (Victório et al. 2010). The presence of terpinen-7-al was not found in plants from Ceará State (Northeast Brazil) and differences in concentrations of main compounds and minor compounds are common in plants grown in different environmental conditions.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC/MS) OF *A. ZERUMBET* ESSENTIAL OIL

The analysis revealed the presence of 19 volatile compounds (Table 1), highlighting the predominance of monoterpenes (98.41%) in the composition of *A. zerumbet* essential oil.

The main constituents as shown in Table 1 are terpinen-4-ol (23.92%), 1,8-cineole (19.01%), γ-terpinene (16.63%) and sabinene (10.14%). A similar composition was shown for the essential oil of *A. zerumbet* in another study from Ceará State, where 6 collections were done during the day and similar composition was observed, only changing the yield among constituents and sabinene was the major constituent (25.4% a 21.48%) (Canuto et al. 2015).

These results contribute to a better understanding of the chemical composition of the plant, opening possibilities for additional investigations into the therapeutic properties and potential applications of *A. zerumbet* essential oil, especially considering the diversity and synergism of the compounds present.

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Table 1. Relative percentage composition of the essential oil from the leaves of *Alpinia zerumbet* by Gas Chromatography/ Mass Spectrometry (GC/MS).

Constituent	IK lit	IK calc	Yield (%)
α-Thujene	911	919	4.06
α-Pinene	932	925	1.59
Sabinene	961	964	10.14
β-Pinene	976	967	2.68
β-Myrcene	981	983	1.26
α-Terpinene	1017	1008	5.67
o-Cymene	1022	1017	4.26
D-Limonene	1029	1021	2.21
Eucalyptol	1030	1023	19.01
γ-Terpinene	1060	1053	16.63
Terpinolene	1078	1084	2.55
cis-Sabinene hydrate	1096	1095	0.59
β-Linalool	1100	1098	0.98
trans-Sabinene hydrate	1101	1120	0.88
(E)-p-Menth-2-en-1-ol	1124	1139	0.58
(-)-Terpinen-4-ol	1177	1180	23.92
α-Terpineol	1188	1194	1.39
β-Caryophyllene	1421	1423	0.99
Caryophyllene oxide	1583	1572	0.60

The Kovats indices (KI) were estimated by linear regression of the retention times of the main compounds in the chromatograms and their respective Kovats indices from the literature (Adams 2017).

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY AND ANTICHOLINESTERASE ACTIVITY

Oxidative stress in relation to Alzheimer's disease has been the subject of extensive research. Although the exact cause of Alzheimer's disease is not fully understood, there is growing evidence suggesting that oxidative stress plays a significant role in its development and progression (Bakari et al. 2015).

These interactions between oxidative stress and Alzheimer's disease suggest that strategies aimed at reducing oxidative stress may play a role in the prevention or treatment of the disease. Studies have explored the potential of antioxidants, both through diet and medications, as possible therapeutic approaches. When the production of reactive oxygen species prevails over the brain defense systems, the lipid-rich constitution of the brain might favor lipid peroxidation in conjunction with defective antioxidant defenses, constituting a free radical chain reaction that may alter overall brain activities (Bouayed et al. 2009). However, it is important to note that the understanding of Alzheimer's disease is complex, involving genetic, environmental, and other factors, and there is still much to be discovered about the causes and effective treatments (Briyal et al. 2023).

Ngameni et al. (2013) proposed that essential oils with IC_{50} < 50 µg mL^{-1} in the assessment of free radical scavenging activity demonstrate high activity, those with IC_{50} <

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100 µg mL^{-1} exhibit moderate activity, and those with IC_{50} >100 µg mL^{-1} show low antioxidant activity. The essential oil of *A. zerumbet* showed relevant activity in scavenging free radicals, leading to the decrease or even the inhibition of these oxidative processes (Alves et al. 2010).

Scientific studies have focused on the antioxidant activity of eucalyptol, highlighting its beneficial properties in different contexts, including the ability to neutralize reactive oxygen species (ROS) and protect against oxidative stress (Yin et al. 2020).

Studies by Picollo et al. (2008) highlighted the high inhibition activity of the enzyme AChE (acetylcholinesterase) by eucalyptol in *in vivo* experiments conducted on lice brains. The essential oil of *Eucalyptus globulus*, composed mainly by eucalyptol (49.07–83.59%) and α-pinene (1.27–26.35%), displays anticholinesterase and anti-inflammatory properties, improves memory, and alleviates symptoms of Alzheimer's disease (Soares et al. 2022).

The influence of terpine-4-ol on the regulation of genes and cellular signaling pathways related to the antioxidant and inflammatory response highlights its role as a multifaceted modulator. Its ability to interfere with oxidation processes and, at the same time, inhibit inflammatory pathways suggests that terpine-4-ol may play an important role in maintaining redox balance and reducing inflammation associated with oxidative stress (Mendes et al. 2022). Furthermore, terpine-4-ol demonstrates the ability to activate endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. This positive modulation of antioxidant enzymes reinforces cellular defense against oxidative stress, enhancing the body's ability to confront and neutralize reactive species (Rocha 2012).

Studies of the *Croton nepetaefolius* essential oil and constituents as anticholinesterase agents revealed, based on molecular docking simulations, that several molecules analyzed have a high interaction potential with the AChE target, highlighting sesquiterpenes germacrene B, followed by ß-caryophyllene with close action to physostigmine; nevertheless, their action is on a different site of AChE structure, being probably non-competitive inhibitors (Passos et al. 2023).

Then, natural products which present antioxidant and anticholinesterase activities are interesting targets for using in AD treatment. The essential oil of *A. zerumbet* displays both activities (Table 2), being a useful source of molecules with potential for AD treatment.

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Table 2. Antioxidant activity (DPPH and ABTS) and anticholinesterase activity of the essential oil of *Alpinia zerumbet* (OEAz).

Amostra	CI_{50} DPPH ($\mu g\ mL^{-1}$)	CI_{50} ABTS ($\mu g\ mL^{-1}$)	CI_{50} AChE ($\mu g\ mL^{-1}$)
OEAz	15.84 ± 0.08	11.27 ± 0.01	15.28 ± 0.01
Quercetin (Standard)	1.63 ± 0.03	1.83 ± 0.02	-
BHT (Standard)	4.75 ± 0.05	4.31 ± 0.02	-
Physostigmine (Standard)	-	-	1.15 ± 0.05

The *A. zerumbet* leaf essential oil present mainly monoterpenes, which are great candidates for the development of new drugs for the treatment of various pathological processes, linked to CNS, including painful conditions. As other example, gamma terpinene (*γ*-TPN) is a monoterpene present in plant species that have multiple pharmacological properties and has structural similarity to antinociceptive monoterpenes, such as limonene and alpha-phellandrene. *γ*-TPN produced antinociceptive effect in models of chemical nociception through the cholinergic and opioid systems involvement (Passos et al. 2015). Then drugs that act on the cholinergic system represent a promising option to treat AD patients (Ferreira-Vieira et al. 2016).

IN SILICO EVALUATION

The marked presence of terpine-4-ol, eucalyptol, γ-terpinene and sabinene is in accordance with literature data for samples from Ceará State, revealed the consistency of these compounds as the main constituents of *A. zerumbet* essential oil (Canuto et al. 2015). It is important to note that even compounds present in reduced quantities in the plant, they can perform biological activities, depending on their action potential and possible synergies with other components present in the oil. Then, in the evaluation of the potential of *A. zerumbet* essential oil for AD, the main constituents and other in lesser concentration completing 10 compounds, were examined *in silico* against AChE three-dimensional structure.

The receptor-ligand complexes formed showed RMSD values ranging from 0.088 to 1.410 Å and affinity energy ranging from -5.8 to -6.8 kcal/mol (Table 3). The redocking of the inhibitor GNT co-crystallized in AChE presented an RMSD of 1.932 Å and affinity energy in the order of -7.9 kcal/mol. Analyzing the interaction patterns with AChE, it was possible to identify that the formed complexes predominantly exhibited Hydrophobic interactions, as well as two Pi-Donor H-Bonds and two Pi-Pi Stacked interactions (Table 3).

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Table 3. Types of interactions and distances (Å) between the ligands and the amino acid residues of AChE

Ligands	Affinity (kcal/mol)	RMSD (Å)	Residue	Interaction	Distance (Å)
α-Terpinene	-6.4	1.410	Trp 286A	Hydrophobic	3.81
			Trp 286A	Hydrophobic	4.02
			Val 294A	Hydrophobic	4.16
			Tyr 337A	Hydrophobic	5.48
			Phe 338A	Hydrophobic	4.87
			Phe 338A	Hydrophobic	4.92
			Tyr 341A	Hydrophobic	4.27
			Tyr 341A	Hydrophobic	5.39
			Trp 286A	Pi-Pi Stacked	4.05
			Trp 286A	Pi-Pi Stacked	5.39
α-Thujene	-6.2	0.992	Val 330A	Hydrophobic	4.47
			Lys 332A	Hydrophobic	5.08
			Val 408A	Hydrophobic	4.23
			Val 429A	Hydrophobic	4.24
			Val 429A	Hydrophobic	4.51
			Val 429A	Hydrophobic	4.56
			Val 429A	Hydrophobic	4.57
			Tyr 510A	Hydrophobic	4.43
			Leu 524A	Hydrophobic	4.76
			Leu 524A	Hydrophobic	5.29
			Arg 525A	Hydrophobic	4.45
			Arg 525A	Hydrophobic	4.46
β-Pinene	-6.2	1.121	Trp 86A	Hydrophobic	3.55
			Trp 86A	Hydrophobic	4.18
			Trp 86A	Hydrophobic	4.25
			Trp 86A	Hydrophobic	4.51
			Trp 86A	Hydrophobic	4.63
			Trp 86A	Hydrophobic	5.07
			Tyr 337A	Hydrophobic	4.58
			Tyr 337A	Hydrophobic	4.79
			Tyr 337A	Hydrophobic	5.18
			Phe 338A	Hydrophobic	5.47
			His 447A	Hydrophobic	4.74
			His 447A	Hydrophobic	5.31
D-Limonene	-6.5	0.150	Val 330A	Hydrophobic	4.33
			Val 408A	Hydrophobic	4.20
			Val 429A	Hydrophobic	3.83
			Arg 525A	Hydrophobic	3.85
			Arg 525A	Hydrophobic	4.85
			Arg 525A	Hydrophobic	4.85
Eucalyptol	-6.4	1.195	Trp 86B	Hydrophobic	3.50
			Trp 86B	Hydrophobic	3.55
			Trp 86B	Hydrophobic	4.03
			Trp 86B	Hydrophobic	4.25
			Trp 86B	Hydrophobic	4.40
			Trp 86B	Hydrophobic	4.81
			Tyr 337B	Hydrophobic	3.84
			Tyr 337B	Hydrophobic	4.94
			Tyr 337B	Hydrophobic	5.14
			Phe 338B	Hydrophobic	5.22
			His 447B	Hydrophobic	4.70
			Tyr 449B	Hydrophobic	5.45
γ-Terpinene	-6.3	0.088	Val 330A	Hydrophobic	4.44
			Lys 332A	Hydrophobic	4.55

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			Val 408A	Hydrophobic	3.95
			Val 429A	Hydrophobic	3.89
			Val 429A	Hydrophobic	3.96
			Val 429A	Hydrophobic	4.71
			Leu 524A	Hydrophobic	4.11
			Arg 525A	Hydrophobic	4.21
			Arg 525A	Hydrophobic	4.96
			Arg 525A	Pi-Donor H-Bond	2.74
o-Cymene	-6.8	1.088	Val 330A	Hydrophobic	4.57
			Lys 332A	Hydrophobic	4.63
			Lys 332A	Hydrophobic	4.94
			Val 408A	Hydrophobic	4.77
			Val 408A	Hydrophobic	5.42
			Val 429A	Hydrophobic	3.58
			Val 429A	Hydrophobic	4.14
			Val 429A	Hydrophobic	4.54
			Arg 525A	Hydrophobic	4.47
			Arg 525A	Pi-Donor H-Bond	2.99
Sabinene	-5.8	1.403	Val 330A	Hydrophobic	4.72
			Lys 332A	Hydrophobic	5.07
			Val 429A	Hydrophobic	3.93
			Val 429A	Hydrophobic	4.18
			Val 429A	Hydrophobic	4.24
			Tyr 510A	Hydrophobic	5.41
			Arg 525A	Hydrophobic	4.53
			Arg 525A	Hydrophobic	4.59
Terpinen-4-ol	-6.5	1.175	Val 330A	Hydrophobic	4.65
			Val 408A	Hydrophobic	4.21
			Val 429A	Hydrophobic	4.11
			Val 429A	Hydrophobic	4.53
			Leu 524A	Hydrophobic	4.34
			Arg 525A	Hydrophobic	4.18
			Arg 525A	Hydrophobic	4.53
Terpinolene	-6.5	1.137	Trp 86B	Hydrophobic	3.46
			Trp 86B	Hydrophobic	3.88
			Trp 86B	Hydrophobic	4.56
			Trp 86B	Hydrophobic	4.82
			Trp 86B	Hydrophobic	5.21
			Tyr 337B	Hydrophobic	3.85

The binding site of the co-crystallized galantamine inhibitor in chains A and B of the AChE receptor is formed by the residues Trp 86, Gly 120, Gly 121, Gly 122, Glu 202, Ser 203, Phe 295, Phe 297, Tyr 337, and His 447 (CHEUNG et al., 2012). It was observed that α-terpinene, β-pinene, 1,8-cineole, and terpinolene bound to the same region as the galantamine binding site, sharing interactions with the residues Tyr 337A (α-terpinene); Trp 86A, Tyr 337A, His 447A (β-pinene); Trp 86B, Tyr 337B, His 447B (eucalyptol); and Trp 86B, Tyr 337B (terpinolene), indicating that these compounds have a similar action to galantamine (Fig. 1). The compound *o*-cymene bound to a different region than the

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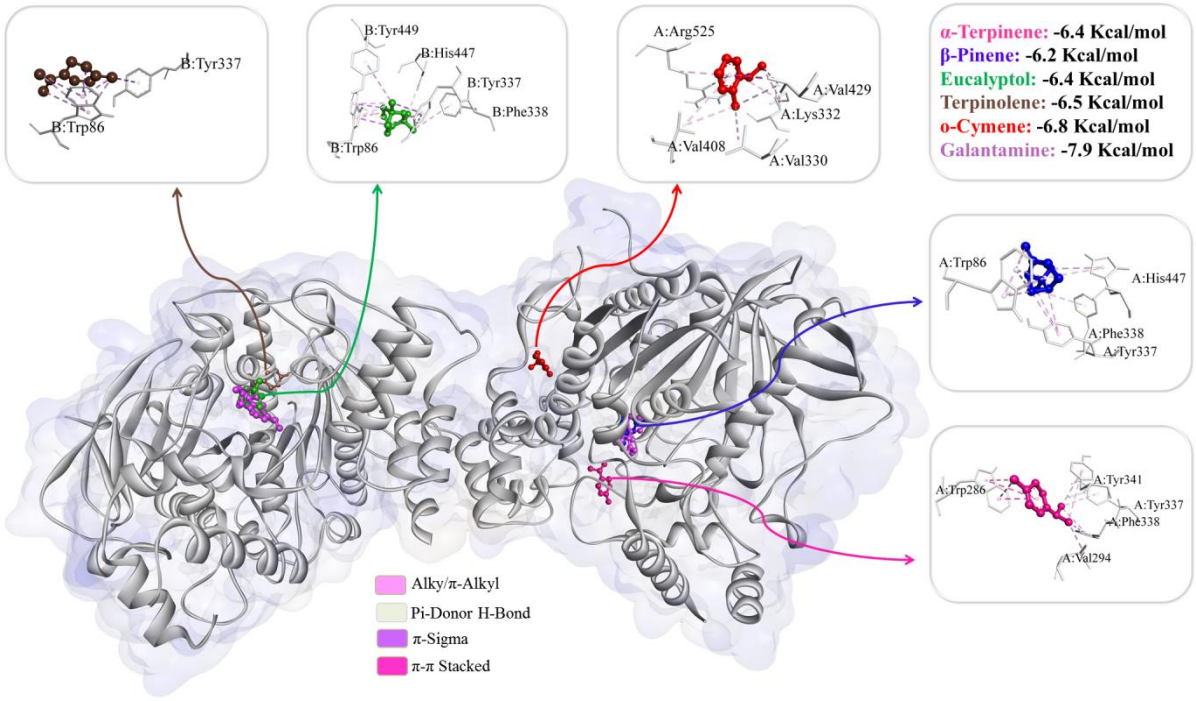
galantamine binding site, indicating a possible synergistic effect with the co-crystallized inhibitor in AChE.

Fig. 1. Interaction complex between acetylcholinesterase (white), α-Terpinene (pink), β-Pinene (blue), Eucalyptol (green), Terpinolene (brown), *o*-Cymene (red), and the co-crystallized inhibitor Galantamine (lilac).



CONCLUSIONS

Through molecular docking studies, it is possible to infer that the compounds α-terpinene, β-pinene, eucalyptol and terpinolene are promising in the planning and development of new anti-Alzheimer's drugs, as they showed significant interactions with AchE, fitting in the same region as the galantamine binding site, indicating similar action.

The data also show that *o*-cymene presented a more favorable affinity energy compared to AChE (-6.8 kcal/mol) and fit in a different region than the galantamine binding site, indicating a possible synergistic effect with the other constituents of the essential oil in the inhibition from AChE.

Then, this study demonstrated that *Alpinia zerumbet* essential oil presented satisfactory results for the presence of antioxidant substances, and for the inhibition of the acetylcholinesterase enzyme, thus indicating the need to carry out other studies in order to confirm its promising pharmacological effects against Alzheimer Disease.

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AUTHOR'S CONTRIBUTION

All the authors have contributed to this work in an appropriate way.

DECLARATIONS

Conflict of interest: The authors declare that they have no confict of interest.

Ethical approval Not Applicable. The datasets and the work do not contain personal or sensitive information; no ethical issue is concerned.

There is no human study in this work.

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