

Original Research Article
**Chemical constitution and bioactivity of the
essential oil from the leaves of
Tapiriraguiensis Aubl**

ABSTRACT

The *Tapiriraguiensis* Aubl species is used in folk medicine to treat leprosy, diarrhea and syphilis. The present study aims to evaluate the chemical constitution and the antioxidant, anticholinesterase and antifungal potential of the essential oil of *T. guianensis* leaves. The plant material was collected at Arco Estadual do Cocó, with prior authorization from the Municipal Environment Department. The essential oil was extracted by the hydrodistillation process with a Clevenger type dispenser and the constituents were evaluated through Gas Chromatography coupled to Mass Spectrometry. The antioxidant potential was evaluated by free radicals: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS+ (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). 96-well plate. Antifungal activity was evaluated using the Clinical Laboratory Standards Institute-M38-A protocol (CLSI, 2018). Gas chromatography coupled with mass spectrometry showed the presence of the constituent eugenol (59.00%), α -copaene (0.40%), β -caryophyllene (29.91%), α -humulene *T. guianensis*. The essential oil showed very promising antioxidant and anticholinesterase potential in vitro studies. Molecular docking in relation to acetylcholinesterase enzyme inhibition revealed that α -copaene has a superior action to the drug physostigmine. The results of molecular docking for *Candida albicans* showed that α -copaene and α -humulene formed a complex in the same binding region of the PepA inhibitor against SAP5, which indicates your actions similar to the aspartic protease inhibitor, in addition to the possibility of a synergistic effect with the drug fluconazole. This is the first study of the biological potential of the essential oil of *T. guianensis* leaves, thus exposing its high potential for future studies in the scientific community. Therefore, we can infer that the essential oil of *T. guianensis* is a source of antioxidant, anticholinesterase and antifungal constituents, with promising therapeutic potential in the management of Alzheimer's disease and *Candida* infections.

Keywords: Essential oil; *Tapiriraguiensis*; Antioxidant; Antifungal; Acetylcholinesterase.

1. INTRODUCTION

The *Tapiriraguiensis* Aubl belongs to the family Anacardiaceae. Species of the genus *Tapirira* range from southern Mexico to South America [1]. *Tapirira obtusa* (Benth.) J.D. Mitch., *T. pilosa* Sprague, *T. retusa* Ducke and *T. guianensis* Aubl., are species of the genus that have already been reported here in Brazilian territory, where it is commonly known as pau-pombo or pigeon chest. In folk medicine, its leaves are widely used to treat leprosy, diarrhea and syphilis [2]. Antioxidant and cholinesterase activities have also been reported for the leaves and stem bark of *Tapiriraguiensis* [3].

Some approaches have been used in individuals with Alzheimer's disease such as the use of antioxidant substances and acetylcholinesterase inhibitors. Acetylcholinesterase inhibitors promote increased levels of the neurotransmitter acetylcholine, enabling more efficient and long-lasting nerve synapses within the cholinergic complex [4]. Moreover, because free

radicals participate in the oxidation of biomolecules and lead to the loss of their biological activities and/or homeostatic imbalance, the use of antioxidant substances is also configured as a therapeutic strategy against neurodegenerative diseases [5-6-7].

Most of

the drugs used in patients with AD do not provide a treatment and/or cure, and the prices of these drugs are high. There is a lack of positive outcomes in patients who use the drugs, bringing with it a high demand for the industry, with respect to the search for new drugs with greater effectiveness and low side effects.

Plants with high levels of phenolic compounds such as *Anacardium occidentale*, *Ceibapentandra*, *Lagunculariaracemosa*, *Mangifera indica*, *Myracrodrumurundeuva* and *Terminaliacatappasho* showed excellent antioxidant activity against the DPPH radical (with IC₅₀ ranging from 3.44±0.16 to 3.73±0.12 µg mL⁻¹) and high power of inhibition of the AChE enzyme (IC₅₀ < 20 µg mL⁻¹) were recommended for more specific studies related to Alzheimer's disease [8].

In recent decades, microbial infections have been on the rise, and with it, morbidity and mortality rates have also increased significantly. Both the resistance patterns of these microorganisms and the proliferation of new pathogens have become a challenge in the eradication of infections. Public health worldwide suffers because of the low effectiveness of the antimicrobial drugs that are used. Several studies have been reported on the fight against pathogenic microorganisms using natural products from plants, animals and microorganisms. Natural products have shown significant efficacy for the treatment of infectious diseases, as well as demonstrating low-intensity side effects, synergy and the potential to overcome drug resistance [9].

Essential oils are of volatile constituents generally of terpenic or aryl propanoid structures that exhibit varied biological activities. The study of essential oil with antimicrobial activities are well known as well as AChE enzyme inhibition [10]. Commercially available essential oils extracted from *Artemisiadracunculus* L., *Inulagraveolens* L., *Lavandula officinalis* Chaix, and *Ocimum sanctum* L. and the components of these oils were screened by microplate assay method to determine their acetylcholinesterase (AChE) inhibitory activity. The results showed that the oils, with exposed activity and among the essential oil components, five components, namely 1,8-cineole, α-pinene, eugenol, α-terpineol and terpinen-4-ol, showed better AChE inhibitory activity, highlighting the constituent eugenol [11].

A practical way to compare the activities of the oils' constituents can be done through a computational theoretical study of the inhibition of enzymes related to the studied diseases. For Alzheimer's disease the enzyme AChE and for fungal infection the enzyme AIS3a products. Based on the docking and molecular dynamics studies of thymol and thymol acetate present in the essential oil of *Lippia thyoides*, these compounds interact with the catalytic residues Ser203 and His447 of the active site of acetylcholinesterase. The free binding energies (ΔG_{bind}) for these ligands were -18.49 and -26.88 kcal/mol, demonstrating that the ligands are able to interact with the protein and inhibit its catalytic activity [63].

The present research aims to qualify the essential oil of the leaves of *Tapiriraguianensis* Aubl. as a source of antioxidant, anti-acetylcholinesterase and antifungal agents, useful in combating the symptoms of Alzheimer's Disease and Candidiasis, through *in vitro* and *in silico* studies.

2. MATERIAL AND METHODS

2.1. Chemicals and Equipment

To perform the tests here, solvents from J.T. Baker – Radnor, USA; Éxodo Científica – São Paulo, BR; and Neon – São Paulo, Brazil. Reagents were purchased from Sigma-Aldrich – St. Louis, USA and Carvalhães – Alvorada, RS, Brazil. The devices used were a Biotek ELISA reader – Vermont, USA; Genesys 10SUV-Vis Spectrophotometer – Thermo Scientific – Vantaa, Finland. The oil analysis was performed on the Shimadzu QP-2010 equipment. The *in silico* assays used the systems: AutoDock Vina, Discovery Studio™ Pymol and UCSF Chimera™.

2.2. Material collection

The plant material was collected from a garden of the Cocó State Park, Fortaleza, Ceará, Brazil. Exsiccates were identified by the botanist Luiz Wilson Lima-Verde and deposited in the Prisco Bezerra Herbarium of the Federal University of Ceará (UFC). The license for the collection of plant material was granted by the State Secretariat for the Environment of Ceará through authorization 04/2021. The essential oil was extracted using about 400g of fresh leaves, through of the hydrodistillation technique in a Clevenger type dispenser.

Table 1. Identification of the species collected in the Cocó State Park in Fortaleza, Ceará, Brazil

Species	Family	Part used	Exsiccate	Coordinates
<i>Tapiriraguianensis</i> Aubl	Anacardiaceae	Leaves	64238	3°74'46.2"S 38°48'78.2"W

2.2.1. Gas Chromatography-Mass Spectrometry (GC/MS)

The oil analysis was performed on the Shimadzu QP-2010 equipment, under the following conditions: Rxt-5MS chromatographic column (Crossbond 5%, diphenyl/95% dimethyl polysiloxane), capillary (30m x 0.25mm x 0.25µm) coated with fused silica; Helium as carrier gas (24.2 mL/min), with constant linear velocity; injector temperature of 250°C (split mode 1:100); detector temperature of 250°C. In addition, the heating ramp was programmed, initially, from 35°C to 180°C, with an increase of 4°C/min to 180°C and, later, 17°C/min to 280°C, remaining at that temperature for the final 10 minutes. Thus, the chromatogram was generated, which relates the relative retention time to the sample peaks.

The mass spectrum was obtained by electronic impact through a beam with 70 eV energy. In this way, mass spectra were generated, whose equipment suggests some compounds, through a comparison with an existing library. To effectively identify the oil components, the following were analyzed: the chromatogram, the mass spectra, the Kovats indexes in the literature according to the NIST and Adams database [13] and the retention time of each compound. The calculation of the experimental Kovats Index was performed using linear regression.

2.2.2. Determination of antioxidant activity by the DPPH Method

The antioxidant potential was measured in 96-well flat-bottomed plates using an Elisa BioTek reader, model ELX800, "Gen5 V2.04.11" software [14] with some modifications. In the 96-well plates, the solutions were used per well: 180 µL of methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), 20 µL of the extracts sampled dissolved in methanol and diluted 10 times to obtain the final concentration 0.2 mg mL⁻¹. Oil concentrations were reproduced using the initial concentration solution of 2 mg mL⁻¹, 200 µg mL⁻¹, 100 µg mL⁻¹, 50 µg mL⁻¹, 25 µg mL⁻¹, 12.5 µg mL⁻¹, 6.25 µg mL⁻¹, 3.12 µg mL⁻¹, 1.56 µg mL⁻¹ and 0.78 µg mL⁻¹. Absorbances were read at 490 nm for a total of 60 minutes of incubation. The standard used for comparison was the BHT. All samples were analyzed in triplicate.

2.2.3. Assessment of Antioxidant Activity by the ABTS Method

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 h. Subsequently, 1 ml of this solution was added to 99 ml of ethanol. The absorbance is read at 734 nm (0.715). Various solutions of decreasing concentrations of *Tapiriraguianensis* essential oil were prepared and 3.0 ml of ABTS^{•+} solution was added to 30 µl of these solutions after 6 min, readings were taken at 734 nm [15]. The IC₅₀ (mean inhibition concentration) was calculated by linear regression.

2.2.4. In vitro evaluation of acetylcholinesterase inhibition

The methodology described by ELLMAN *et al.* (1961) with some modifications was used to evaluate the inhibition of the essential oil against the enzyme acetylcholinesterase with modifications [16]. Acetylcholinesterase inhibition potential was verified in 96-well flat-bottomed plates with a BioTek ELISA reader, model ELX800, software "Gen5 V2.04.11". The reagents used per well were: 25 µL of acetylthiocholine iodide (15 mM), 125 µL of 5,5'-dithiobis-[2-nitrobenzoic] in 0.1 M Tris/HCl NaCl and 0.02 M MgCl₂ solution. 6 H₂O, 50 µL Tris/HCl solution with 0.1% bovine serum albumin, 25 µL samples/standards. Physostigmine (Eserine) and Galanthamine patterns were evaluated separately with 25 µL of AChE (0.22 unit, µL⁻¹). The evaluation was analyzed in triplicate.

2.2.5. Determination of antifungal activity

Antifungal activity was measured according to the method described by Fontenelle et al. (2007) using broth microdilution MIC tests, based on the Clinical Laboratory Standards Institute protocol M38-A/2018. *Candida albicans* fungal strains 0131, 0128, 0102 and 0104 obtained from the mycoteca of Federal University of Pernambuco were used [17-18]. The MIC was determined in 96-well microplates in which 10 mg mL⁻¹ of diluted extract 50 µL of 5% DMSO and 950 µL of RPMI medium and 50 µL of RPMI medium were added to all wells of the first column followed by addition of a series of dilutions 0.002 to 2.5 mg mL⁻¹ and 100 µL of the inoculum. The plates were incubated at 37 °C and visual reading was performed after 48 hours. The positive control used was fluconazole. The assays were performed in duplicate, and the MIC was defined as the lowest concentration of sample capable of inhibiting 100% of the visible growth of the microorganism. Results were determined by visualization as recommended by CLSI. The minimum fungicidal concentration (MFC) was determined by subculturing 100 µL of solution removed from wells, without turbidity, on potato dextrose agar at 28 °C and determined as the lowest concentration that resulted in no growth in the subculture after 48 hours.

2.2.6. Molecular docking - In silico evaluation of acetylcholinesterase inhibition and anti-Candida activity

The chemical structures of the ligands acetyl eugenol (CID7136), α -copaene (CID19725), α -humulene (CID5281520), β -caryophyllene (CID5281515),

Eugenol (CID3314) and the drug physostigmine (CID5983) were imported from the PubChem repository (<https://pubchem.ncbi.nlm.nih.gov/>), these saved at physiological pH using the Marvin Sketccch code [19]. The mechanisms of action of ligands against acetylcholinesterase (AChE) were analyzed in silico using molecular docking simulations [12]. The target structure was imported from the Protein Data Bank (<https://www.rcsb.org/>), identified as "Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with (-)-galantamine" (PDB ID:4EY6) [20].

50 independent simulations were run using the AutoDock Vina code [21], configured to run the Lamarckian Genetic Algorithm (LGA) and the Exhaustiveness 64 algorithm [22]. To validate the simulations performed, the redocking technique was performed with the drug Galantamine (GNT) co-crystallized in the AChE target. TM [23]. To validate the coupling simulations in the evaluation against *C. albicans*, the SAP5 receptor, identified as "*C. albicans* secreted aspartic protein (Sap) 5" (PDB 2QZX) was used. Results were analyzed using Discovery Studio TM [62], Pymol [61] and UCSF Chimera TM [23] codes. To validate the docking simulations, the redocking technique was performed with the co-crystallized inhibitor PepA (SAP5). [24]. Residues were removed, polar hydrogens added and Gasteiger charges calculated [25] using the Autodock tools TM code [26].

The lowest energy conformers were optimized using Avogadro [27], configured to use the steepest descent algorithm with 50 iteration cycles, applying MMFF94 (Merck Molecular Force Field [28-29]). The statistical parameter RMSD (Root to Mean Square Deviation) up to 2.0 Å [30] and the affinity energy with values lower than -6.0 kcal/mol [12-31] were used as a criterion to select the best pose.

The strength of the hydrogen bonds (H bond) was analyzed through the values of the distances between the donor and acceptor atoms, classified as Strong bonds when they present distances between 2.5-3.1 Å, Medium bonds between 3.1-3.5 Å and Weak bonds when they have a distance greater than 3.55 Å [32].

3. RESULTS AND DISCUSSION

3.1. CHEMICAL CONSTITUTION OF THE ESSENTIAL OIL OF TAPIRIRA GUIANENSIS

The percentage of compounds identified in the essential oil from the leaves are listed in Table 2 along with their experimental and literature retention indices. The oil showed a yield of 0.15%, corroborating with the reported range 0.13% - 0.24% in the literature by [33]. Among the constituents identified in the present study: α -copaene, β -caryophyllene and α -humulene were common with the study found.

Table 2. Relative percentage composition of the essential oil of *T. guianensis* leaves by gas chromatography-mass spectrometry (GC-MS)

Constituintes	KI(lit)	KI(exp)	%	Zoghbi et al 2014 (KI)
Eugenol	1373	1367	59,00	-
α -copaene	1376	1382	0,40	1380

β -cariophyllene	1417	1423	29,91	1417
α -humulene	1452	1455	3,17	1452
Acetyeugenol	1524	1523	7,52	-
Total			100	

Kovats indexes (KI) were estimated by linear regression of retention times of main compounds in the chromatograms and respective Kovats index from the literature [13].

Eugenol was the majority compound identified in the essential oil of *T. guianensis* leaves and is classified as a phenylpropanoid. It is considered the main phenolic compound in clove essential oil, so it is obtained from the shoots and leaves of *Eugeniacaryophyllata* [64-65]. The constituent acetyeugenol is classified as a vanilloid. It exhibits antioxidant property that has already been reported. Diets rich in both eugenol and acetyeugenol may reduce the risks of diseases such as cancer, cardiovascular disorders, malaria, AIDS, and the effects of aging [34-35-36].

The constituent α -copaene is classified as a tricyclic sesquiterpene. It has a range of applications across the food, drug, and agricultural industries [37-66]. The constituent β -caryophyllene was the second major constituent of the essential oil from *T. guianensis* leaves. It is considered as a sesquiterpene widely found in essential oils of spices such as black pepper, cinnamon and oregano and also in various plants, mainly *Cannabis sativa* and *Copaifera* spp. [38].

The constituent α -humulene is classified as a naturally occurring monocyclic sesquiterpene. It is one of the constituents of the essential oil of the flowering cone of the hop plant, *Humulus lupulus*, from which its name comes [39]. α -Humulene or α -Caryophyllene ((1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene) contains in its structure an eleven-membered ring containing three trans-endocyclic (1-2,4-5 and 8-9) double bonds, where two are double substituted [40-41-42].

3.2. Antioxidant and cholinesterase potential in vitro

The potential of antioxidant agents to eliminate DPPH radicals is commonly attributed to their hydrogen-donating ability. The reaction of DPPH with antioxidants is influenced by the structural conformation of the antioxidant compounds. In addition, the amount of hydroxyls in some substances can provide faster reactions against the DPPH radical [67]. Phenolic substances are already well described in the literature as excellent antioxidant agents [43]. The ortho-dihydroxylated [44-45] and para-dihydroxylated [46] positions in phenolic compounds provide a more pronounced antioxidant efficiency, even though the mechanisms of action are not well defined.

There is a correlational relationship already mentioned by several authors in relation to the antioxidant potential and phenolic compounds. A study with 18 medicinal plants showed that plants with higher levels of total phenols promoted better antioxidant effects by the DPPH (1,1-diphenyl-2-picrylhydrazyl) test [47]. Another study evaluated the relationship of phenolic compounds and antioxidant activity of 30 plants from Coclé State Park in Fortaleza, Ceará. A linear relationship was observed for 10 plants: *A. occidentale*, *C. pentandra*, *H. stigonocarpa*, *L. racemosa*, *L. ferrea*, *M. indica*, *M. tenuiflora*, *M. urundeuva*, *S. mombim*, *T. catta*, with the content of total phenols in the range of $297.46 \pm 26.94 \mu\text{g}\cdot\text{mL}^{-1}$ to $599.30 \pm 17.08 \mu\text{g}\cdot\text{mL}^{-1}$ with antioxidant activities with IC₅₀ for the DPPH radical ranging from 3.44 ± 0.16 to $3.73 \pm 0.12 \mu\text{g}\cdot\text{mL}^{-1}$, respectively [8]. Analyzing the antioxidant potential of the essential oil from the leaves of *T. guianensis*, one can notice a very promising action against the two radicals tested (Table 3).

Table 3. Antioxidant and anticholinesterase activity of the essential oil from the leaves of *T. guianensis* Aubl.

	DPPH IC ₅₀ ($\mu\text{g}/\text{mL}$)	ABTS ⁺ IC ₅₀ ($\mu\text{g}/\text{mL}$)	ACHE IC ₅₀ ($\mu\text{g}/\text{mL}$)
Essential oil	4.39 \pm 0.076	5.24 \pm 0.023	12.56 \pm 0.012
BHT	1.61 \pm 0.04	0.95 \pm 0.06	-

BHT:butylatedhydroxytoluene(Standard);Physo:physostigmine(Standard)

Eugenol, which is presented as the majority constituent (59.00%), should possibly have a strong influence on the antioxidant action. The ability of eugenol to sequester free radicals has already been described in the DPPH assay ($IC_{50}=11.7\mu\text{g/mL}$), as well as its inhibition on reactive oxygen species (ROS) ($IC_{50}=1.6\mu\text{g/mL}$), H_2O_2 ($IC_{50}=22.6\mu\text{g/mL}$ and $27.1\mu\text{g/mL}$) and NO ($IC_{50}<50.0\mu\text{g/mL}$) [48]. With this, we can justify the potential of *T. guianensis* essential oil from the eugenol content in the composition. Although eugenol is the major constituent of *T. guianensis* essential oil and it has a high influence on the ability to eliminate free radicals, the constituents α -copaene, β -caryophyllene and α -humulene also participate in the biological action, since their antioxidant activities have already been proven by other authors.

Regarding the anticholinesterase action (Table 3), the essential oil from *T. guianensis* leaves also showed a very promising action. Alzheimer's disease is characterized as a neurodegenerative pathology that affects thinking, memory, learning, and behavior of affected individuals. Acetylcholinesterase is an enzyme that acts in the termination of cholinergic signaling by hydrolysis of acetylcholine. With this, inhibition of the acetylcholinesterase enzyme may be a very promising strategy in the treatment and management of the disease [49-50].

Using Ellman's spectrophotometric method, the constituent eugenol and five derivatives: 2-Methoxy-4-(oxiran-2-ylmethyl)phenol, 4-(2-Hydroxy-3-(2-hydroxyphenoxy)propyl)-2-methoxyphenol, 4-(2-Hydroxy-3-(3-hydroxyphenoxy)propyl)-2-methoxyphenol, 4-(2-Hydroxy-3-(4-hydroxyphenoxy)propyl)-2-methoxyphenol and 3-(2-Hydroxy-3-(4-hydroxy-3-methoxyphenyl)propoxy)naphthalen-2-ol were analyzed for their action in inhibiting the enzyme acetylcholinesterase. The study showed that all compounds showed promising action against the enzyme with KI values ranging from 90.10 ± 0.01 - $379.57\pm 0.14\text{nM}$ [51].

In some neurodegenerative diseases, the use of β -caryophyllene acts in preventing neuronal death in models of focal ischemia [52], vascular dementia [53], Parkinson's disease [52-54] and Alzheimer's disease [55-56]. We can suggest that the constituent eugenol and β -caryophyllene influence the anticholinesterase action as there are already records concerning their cholinesterase effect. All the constituents recorded in the essential oil of *T. guianensis* leaves were reevaluated *in silico* against the acetylcholinesterase enzyme and will be demonstrated in the topic (*In silico* evaluation of acetylcholinesterase enzyme inhibition).

3.3. Determination of antifungal activity

Through *in vitro* analysis it was possible to demonstrate that the essential oil from *T. guianensis* leaves has an action against *Candida albicans*. The test evaluated the effect of the oil against four strains of *Candida albicans*: 0131 (clinic), 0128 (clinic), 0102 (clinic) and 0104 (clinic). The MIC values ranged from 156 to 312 $\mu\text{g/mL}$ and MFC from 312 to 625 $\mu\text{g/mL}$ (Table 4). Sartoratto and collaborators (2004) classified the antifungal activity of aromatic plants used in Brazil [57]. According to the classification: FCMs lower than 500.0 $\mu\text{g/mL}$ present strong activity; MICs between 500.0-1500.0 $\mu\text{g/mL}$ promote moderate activity and MICs above 1500.0 $\mu\text{g/mL}$ low activity. The MICs found for the strains in the present study show strong antifungal activity of *T. guianensis* essential oil.

Table 4. Anti-*Candida* potential of the essential oil from the leaves of *T. guianensis*

Samples	Strains	MIC ($\mu\text{g/mL}$)	MFC ($\mu\text{g/mL}$)
OE	0131 (clínica)	156	312
FLZ		1	1

OE	0128(clínica)	312	625
FLZ		0.25	0.25
OE	0102(clínica)	156	312
FLZ		0,25	0,25
OE	0105(clínica)	156	312
FLZ		0.25	0.25

MIC:MinimumInhibitoryconcentration;MFCMinimumfungicidalconcentration;FLZ:Fluconazole(Standard)andOE:essenti aloil

The antifungal action of eugenol against *C. albicans* has already been evaluated *in vitro* models. Its activity may be related to the alteration of the cell membrane and cell wall structure, leading to the release of the cell contents [58]. Some authors state that the antifungal action may be related to plasma membrane instability, even denaturation of cytoplasmic proteins, with the ability to inactivate enzymes, causing cell death [59]. β -caryophyllene tested alone, already shows inhibitory action on fungal development [60]. Through the mentioned studies on eugenol and β -caryophyllene, we can justify that the antifungal potential of *T. guianensis* essential oil is due to the presence and contents of these constituents.

3.4. Molecular docking-evaluation of *in silico* results - *In silico* evaluation of acetylcholinesterase enzyme inhibition

The observed RMSD values presented variations from 0.994 to 1.932 Å. With respect to affinity energy, the receptor-ligand complexes formed exhibited values in the range of -6.2 to -8.2 kcal/mol (Table 5).

Table 5. RMSD and affinity energy values calculated in molecular docking simulations

Ligand	Affinity Energy (kcal/mol)	RMSD (Å)
Acetyl eugenol	-7.0	1.435
α -copaene	-8.2	1.076
α -Humulene	-7.8	1.114
β -cariophyllene	-7.7	0.994
Eugenol	-6.2	1.050
Physostigmine	-8.0	1.733
Galantamine*	-7.9	1.932

*Ligand co-crystallized (redocking)

Analyzing the interaction patterns against AChE (Table 6), it was possible to identify that the acetyl eugenol/AChE complex is formed by two hydrophobic interactions, one with the apolar side chain of the

aromatic residue Trp 86^a (4.12 Å) and one with the basic side chain residue His 447A (4.90 Å), two H-bond strong interactions with the uncharged polar side chain residues Tyr124A (2.45 Å), Ser125A (2.12 Å) and a T-shaped Pi-Pi interaction with the apolar side chain of the residue Trp 86A (5.06 Å). The α -Copaene/AChE complex is formed by eight hydrophobic interactions, six with the apolar side chain of residues Trp 86A (3.72, 3.95, 4.17, 4.44 and 4.86 Å), Phe 338A (5.23 Å), one with the uncharged polar side chain residue Tyr 124 (5.32 Å) and one with the basic side chain residue His 447A (4.81 Å). The α -Humulene/AChE complex is formed by five hydrophobic interactions, two with the apolar side chain residue Trp 86B (3.86 and 4.10 Å), two with the uncharged polar side chain residues Tyr 124B (4.62 Å), Tyr 337B (4.80 Å) and one with the basic side chain residue His 447B (5.40 Å). The beta-Caryophyllene/AChE complex is formed by seven hydrophobic interactions, four with the apolar side chain residues Trp 86B (4.63 and 5.28 Å), Phe 338B (4.93 and 5.44 Å), two with the uncharged polar side chain residue Tyr 337B (4.27 and 4.75 Å) and one with the basic side chain residue His 447B (4.65 Å). Eugenol/AChE is formed by four hydrophobic interactions, two with the apolar side chain residue Trp 86A (4.01 and 4.39 Å), two with the uncharged polar side chain residues Tyr 337A (3.61 Å), Tyr 449A (5.15 Å), one H-bond average with the uncharged polar side chain residue Gly 121A (3.50 Å), one H-bond weak with the uncharged polar side chain residue Gly 120A (3.60 Å), two Pi-Pi Stacked interactions with the apolar side chain of the aromatic residue Trp 86 (4.20 and 4.84 Å) and an Unfavorable Donor-Donor interaction with the uncharged polar side chain residue Ser125 (1.11 Å). Physostigmine/AChE is formed by five hydrophobic interactions, four with the apolar side chain residue Trp 86B (3.53, 4.09, 4.20 and 4.30 Å), one with the uncharged polar side chain residue Tyr 337B (4.24 Å), one H-bond strong with the uncharged polar side chain residue Tyr124B. Therefore, these sesquiterpenes α -copaene, α -humulene, and beta-caryophyllene were shown to be the most active against the AChE enzyme by having the best affinity energies.

Table 6. Types of interactions and distances (Å) between ligands and amino acid residues of AChE.

Ligands	Residue	Interaction	Distance (Å)
Acetyeugenol	Trp86A	Hydrophobic	4.12
	His 447A	Hydrophobic	4.90
	Tyr124A	H-Bond	2.45
	Ser125A	H-Bond	2.12
	Trp86A	Pi-Pi T-shaped	5.06
α -copaene	Trp86A	Hydrophobic	3.72
	Trp86A	Hydrophobic	3.95
	Trp86A	Hydrophobic	4.17
	Trp86A	Hydrophobic	4.44
	Trp86A	Hydrophobic	4.86
	Tyr124A	Hydrophobic	5.32
	Phe338A	Hydrophobic	5.23
	His 447A	Hydrophobic	4.81
α -humulene	Trp86B	Hydrophobic	3.86
	Trp86B	Hydrophobic	4.10
	Tyr124B	Hydrophobic	4.62
	Tyr337B	Hydrophobic	4.80
	His447B	Hydrophobic	5.40
β -cariophyllene	Trp86B	Hydrophobic	4.63
	Trp86B	Hydrophobic	5.28

	Tyr337B	Hydrophobic	4.27
	Tyr337B	Hydrophobic	4.75
	Phe338B	Hydrophobic	4.93
	Phe338B	Hydrophobic	5.44
	His 447B	Hydrophobic	4.65
Eugenol	Trp86A	Hydrophobic	4.01
	Trp86A	Hydrophobic	4.39
	Tyr337A	Hydrophobic	3.61
	Tyr449A	Hydrophobic	5.15
	Gly120A	H-Bond	3.60
	Gly121A	H-Bond	3.50
	Trp86A	Pi-PiStacked	4.20
	Trp86A	Pi-PiStacked	4.84
	Ser125A	UnfavorableDonor-Donor	1.11
Physostigmine	Trp86B	Hydrophobic	3.53
	Trp86B	Hydrophobic	4.09
	Trp86B	Hydrophobic	4.20
	Trp86B	Hydrophobic	4.30
	Tyr337B	Hydrophobic	4.24
	Tyr124B	H-Bond	2.40
	Glu 202B	H-Bond	3.58
	Phe338B	Pi-PIT-shaped	5.78

The binding site of Galantamine (GNT) co-crystallized between the A and B chains of the AChE receptor is formed by residues Trp 86, Gly 120, Gly 121, Gly 122, Glu 202, Ser 203, Phe 295, Phe 297, Tyr 337 and His 447 [24]. The evaluated compounds complex in the same region of the Galantamine binding site, having in common interactions with residues Trp 86A and His 447A (acetyl eugenol and α -copaene); Trp 86B, Tyr 337B and His 447B (α -humulene and β -caryophyllene); Trp 86A, Gly 120A, Gly 121A, Tyr 337A (eugenol).

Compared to the drug physostigmine, we observed that acetyl eugenol, α -copaene and eugenol do not compete for the physostigmine binding site, suggesting a possible synergistic effect with physostigmine and similar action to GNT (Chain A). We also observed that α -humulene and β -caryophyllene compounds complex in the same region of the physostigmine binding site, having in common interactions with residues Trp 86B, Tyr 124B and Tyr 337B (α -humulene), Trp 86B, Tyr 337B and Phe 338B (β -caryophyllene), besides binding in the same region of the GNT site (Chain B), indicating that these compounds have similar action to the controls used. Figure 1 shows the most stable receptor-ligand complexes (lowest affinity energy).

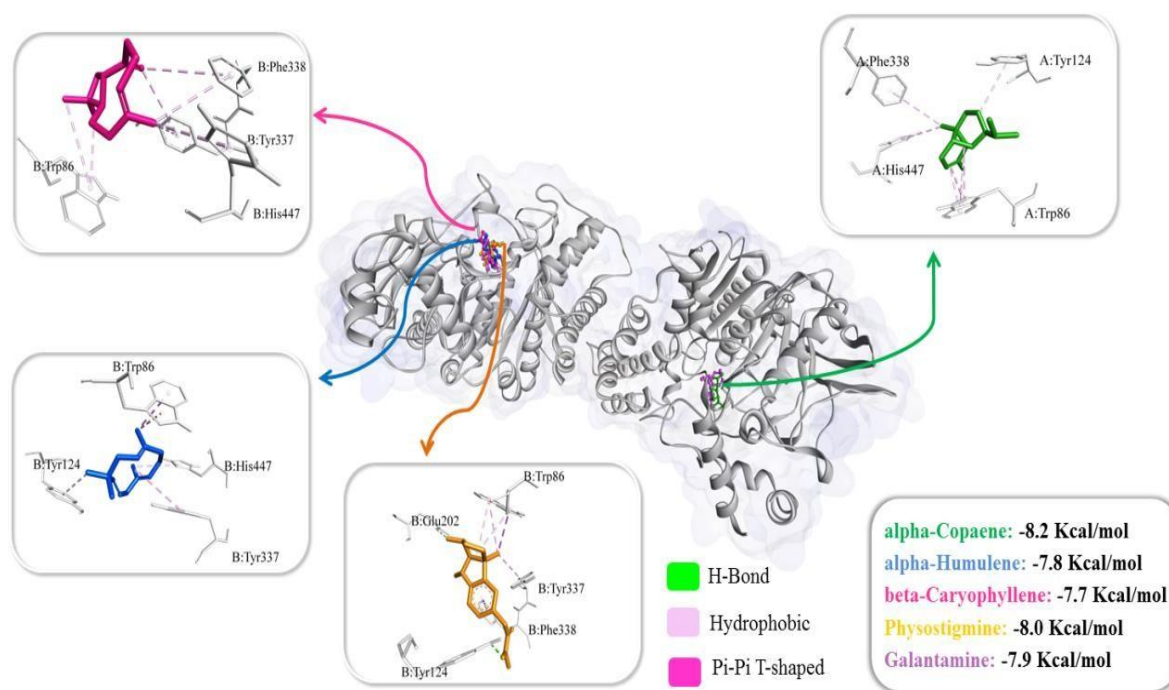


Fig.1. Complex interaction between AChE, α -copaene (green), α -humulene (blue), β -caryophyllene (pink), physostigmine (orange) and the co-crystallized inhibitor galantamine (purple).

3.5. *In silico* evaluation of the anti-*Candida* potential

All binders showed RMSD values within the ideal range with values lower than 2.0 Å, ranging from 0.827 to 1.777 Å with SAP5, statistically validating the simulations performed. With SAP5, it was observed that α -copaene (-7.0 kcal/mol), α -humulene (-6.0 kcal/mol) and β -caryophyllene (-6.2 kcal/mol) ligands presented values within the ideal range for energy of affinity (Table 7). This enzyme was not suitable for a more general evaluation of the activity against fungi since it did not find the well known antifungal action of eugenol [10].

Table 7. Affinity energy values and RMSD of complexes formed with *Candida albicans*

SAP5/Ligand	Energy (kcal/mol)	RMSD (Å)
Acetyeugenol	-5.8	1.268
α -copaene	-7.0	0.827
α -Humulene	-6.0	1.730
β -caryophyllene	-6.2	1.103
Eugenol	-5.7	1.777
Fluconazole	-7.2	1.676
PepA [*] -Ligand co-crystalized (redocking)	-8.0	1.635

Against SAP5 (Table 8), the SAP5/acetyeugenol complex showed hydrophobic interactions with residues Ile 30A, Ile 123A, Tyr 84A, two H-bonds with residues Gly 85A, Asp 86A and a Pi-Pi Stacked interaction with Tyr 84A. SAP5/ α -copaene showed hydrophobic interactions with residues Ile 12A, Ile 30A, Tyr 84A, Ala 119A, Arg 120A and Ile 123A. SAP5/ α -humulene showed hydrophobic interactions with residues Ile 30B, Tyr 84B, Arg 120B and Ile 123B. The SAP5/ β -

caryophyllene complex showed three hydrophobic interactions with the residue Phe 128B. SAP5/Eugenol showed hydrophobic interactions with residues Ala162A, Lys257B, Phe281A and H-bonds with residues Ser277A, Glu278A. SAP5/fluconazole showed a hydrophobic interaction with Ala 162A, an H-bond with Gln282A and two Pi-Sulfur interactions with residues Cys256A, Cys294A.

Table 8. Types of interactions and distances (Å) between ligands and amino acid residues of SAP5 *Candida albicans*.

Ligands	Residue	Interaction	Distance (Å)
Acetyeugenol	Ile30A	Hydrofobic	3.80
	Ile123A	Hydrofobic	4.15
	Tyr84A	Hydrofobic	2.71
	Gly85A	H-Bond	2.36
	Asp 86A	H-Bond	2.63
	Tyr84A	Pi-Pi Stacked	5.03
α -Copaene	Ile12A	Hydrofobic	5.31
	Ile30A	Hydrofobic	4.72
α -Humulene	Ile30A	Hydrofobic	4.86
	Ile30A	Hydrofobic	5.46
	Tyr84A	Hydrofobic	4.39
	Tyr84A	Hydrofobic	4.43
	Tyr84A	Hydrofobic	5.13
	Ala119A	Hydrofobic	4.12
	Ala119A	Hydrofobic	5.28
	Arg120A	Hydrofobic	3.79
	Ile123A	Hydrofobic	5.35
	Ile30B	Hydrofobic	5.25
	Tyr84B	Hydrofobic	4.05
	Arg120B	Hydrofobic	4.20
	Ile123B	Hydrofobic	5.22
β -caryophyllene	Phe128B	Hydrofobic	2.80
	Phe128B	Hydrofobic	4.53
	Phe128B	Hydrofobic	5.15
Eugenol	Ala162A	Hydrofobic	4.83
	Lys 257B	Hydrofobic	4.39

	Phe281A	Hydrophobic	4.21
	Ser277A	H-Bond	2.76
	Glu 278A	H-Bond	2.31
	Glu 278A	H-Bond	2.56
	Glu 278A	H-Bond	3.07
Fluconazole	Ala162A	Hydrophobic	5.49
	Gln282A	H-Bond	2.25
	Cys256A	Pi-Sulfur	5.78
	Cys294A	Pi-Sulfur	5.89

Regarding SAP5, we observed that α -Copaene, α -Humulene and β -caryophyllene ligands formed the most stable receptor-ligand complexes (Figure 2). The SAP5/ α -Copaene complex is formed by hydrophobic interactions with the apolar side chain of residues Ile 12A, Ile30A, Ala119A, with the uncharged polar side chain of residue Tyr84A, and with the basic side chain of residue Arg120A. SAP5/ α -Humulene is formed by hydrophobic interactions with the apolar side chain of residues Ile30B, Ile123B, the uncharged polar side chain of residue Tyr84B, and the basic side chain of residue Arg120B. SAP5/ β -caryophyllene is formed by hydrophobic interactions with the apolar side chain of the Phe residue 128B. SAP5/Fluconazole (control) is formed by a hydrophobic interaction with the apolar side chain of the Ala residue 162A, a Strong H-bond with the non-polar side chain residue charged Gln 282A (2.25 Å) and two Pi-Sulfur interactions with the uncharged polar side chain residues Cys 256A and Cys 294A. The active site of the Sap5/PepA complex is formed by residues Ile12, Asp32, Gly34, Ser35, Lys83, Tyr84, Gly85, Asp86, Ile123, Gly 220, Thr 221, Thr 222, Ile 223 and Ile 305 [24]. Interaction analysis showed that α -copaene and α -humulene interact with amino acid residues of the active site of the Sap5/PepA complex (α -Copaene, Ile 12A, Tyr 84A and Ile 123A; α -Humulene, Tyr 84B and Ile 123B), which indicates a similar action to the PepA inhibitor and the possibility of a synergistic effect with Fluconazole. β -caryophyllene interacts at a different binding site than Pepstatin A and the control, which indicates a possible synergistic effect with PepA and Fluconazole.

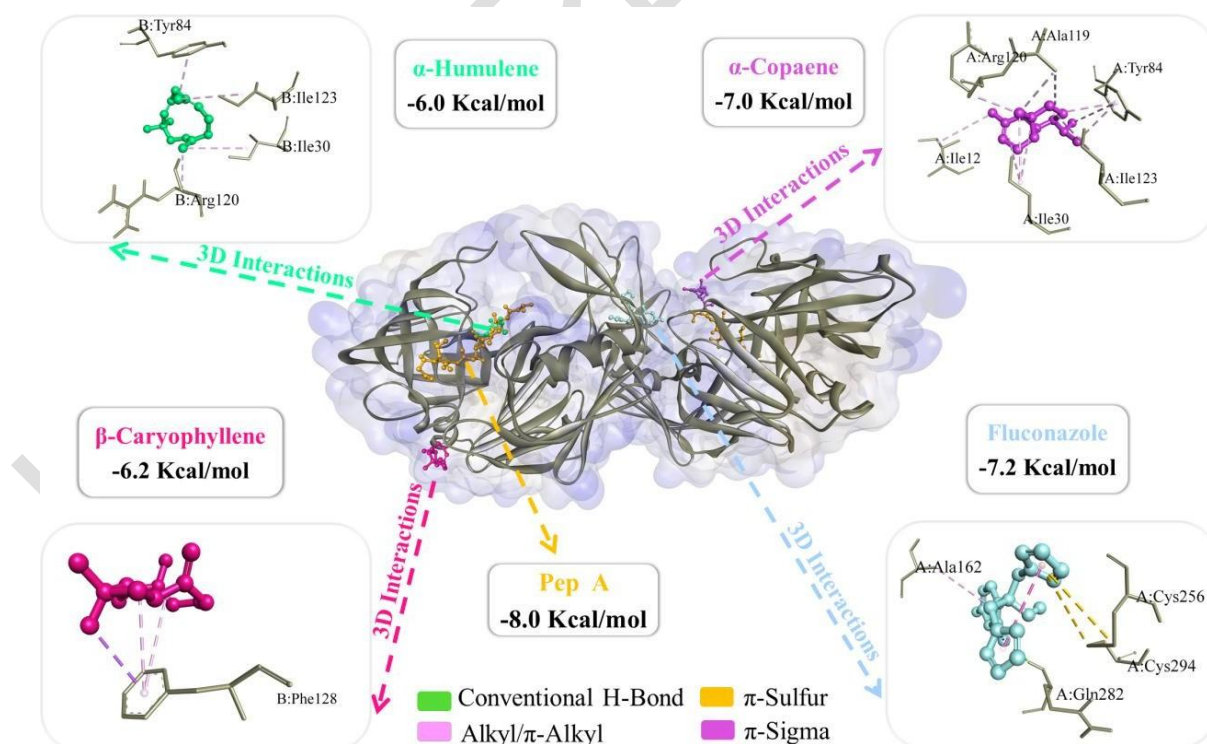


Fig.2. Interaction complex between SAP5, α -Copaene, α -Humulene, β -caryophyllene, Fluconazole and the co-crystallized inhibitor Pepstatin A (PepA).

4. CONCLUSION

T. guianensis leaves show very promising antioxidant, anticholinesterase and antifungal activities, according to *in vitro* analyzes. *In vitro* tests showed promising actions of *Tapiriraguianensis* essential oil for antioxidant, anticholinesterase and anti-*Candida* activity. Eugenol and β -caryophyllene as major constituents are well cited in the literature for having several actions such as: antioxidant, anticholinesterase and antifungal. The *in silico* evaluation for the inhibition of the acetylcholinesterase enzyme showed that all the complexes formed showed hydrophobic interactions with the amino acid residue Trp 86, a residue present in the GNT binding site, indicating that all the ligands evaluated have a similar action to the drug galanthamine. The molecular docking results showed that the sesquiterpene compounds α -copaene, α -humulene and β -caryophyllene have similar or superior action to the drug physostigmine, and also suggest a possible synergistic effect of α -copaene, α -humulene and β -caryophyllene compounds to physostigmine.

The results of molecular docking for *Candida albicans* showed that α -copaene and α -humulene complexed in the same binding region of the PepA inhibitor against SAP5, which indicates its similar action to the aspartic protease inhibitor, in addition to the possibility of a synergistic effect with the drug Fluconazole. We also highlight that β -caryophyllene interacted with the assessed target, complexing at a different site than the co-crystallized PepA inhibitor (SAP5) and the Fluconazole control, which allows us to infer its possible synergistic effect with Fluconazole as a potential tool in the treatment of

fungal infections caused by *Candida albicans*. However, this protein target was not able to demonstrate the activity of the known anti-fungal eugenol.

Therefore, the study qualifies the essential oil from the leaves of *T. guianensis* Aubl. as a source of antioxidant compounds, anticholinesterase that can be explored as therapeutic strategies against Alzheimer's disease and a source of antifungal substances against *Candida albicans* infections.

CONSENT (WHEREEVER APPLICABLE)

Not applicable to this submission.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not applicable to this submission.

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