CG-MS, Radical Scavenging, Acetylcholinesterase Inhibition, Antifungal and Molecular Docking Studies of Essential Oil from the Leaves of *Tapirira guianensis* Aubl

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Authors' contributions

This work was carried out in collaboration among all authors. Author DPdO played a central role in coordinating the collection, processing of plant material, and conducting qualitative and quantitative phytochemical analyses. Meanwhile. Authors LSF and SICGB focused on evaluating the inhibition of the acetylcholinesterase enzyme, and author FFSL was responsible for identifying relevant compounds. The significant contribution of author JRGN in material processing consolidated the experimental foundation of the study. The steps of molecular docking were carried out with expertise by authors MMM, ESM and JS. Authors JCSP and ROdSF led the antifungal analysis, complementing the multifaceted approach of this work. Author SMdM played a crucial role in guiding, supervising, and revising the manuscript, ensuring the cohesion and integral quality of the study. The synergistic collaboration among the authors reflects a steadfast commitment to scientific excellence and the comprehensiveness of the proposed research. All authors read and approved the final manuscript.

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Original Research Article	

ABSTRACT

The species *Tapirira guianensis* Aubl is used in folk medicine to treat leprosy, diarrhea, and syphilis. The present study aims to evaluate the chemical composition and the antioxidant, anticholinesterase, and antifungal potential of the essential oil from 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 through the hydrodistillation process with a Clevenger-type apparatus, and the constituents were evaluated through Gas Chromatography coupled to Mass Spectrometry (GC-MS). The antioxidant potential was assessed through free radicals: DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS+ (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) in a 96-well plate. Antifungal activity was evaluated using the Clinical Laboratory Standards Institute - M38-A protocol (CLSI, 2018). Gas chromatography coupled with mass spectrometry revealed the presence of constituents such as eugenol (59.00%), α-copaene (0.40%), β-caryophyllene (29.91%), α-humulene in T. guianensis. The essential oil demonstrated very promising antioxidant and anticholinesterase potential in *In vitro* studies. Molecular docking regarding acetylcholinesterase enzyme inhibition revealed that α-copaene has superior action compared to the drug physostigmine. The results of molecular docking for *Candida albicans* showed that α-copaene and α-humulene interacted in the same binding region as the PepA inhibitor against SAP5. This indicates their action is 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 from T. guianensis leaves, thus revealing 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.

1. INTRODUCTION

The *Tapirira guanensis* Aubl belongs to the family Anacardiaceae. Species of the genus *Tapirira* range from southern Mexico to South America [1]. *Tapirira obtusa* (Benth.) JD 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 *Tapirira guianensis* [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 (Alzheimer's Disease) 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*, *Ceiba pentandra*, *Laguncularia racemosa*, *Mangifera indica*, *Myracrodrum urundeuva* and *Terminalia catappa* 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 *Artemisia dracunculus* L., *Inula graveolens* 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 infections the enzyme AlS3 adducts. Based on the docking and molecular dynamics studies of thymol and thymol acetate present in the essential oil of *Lippia thymoides*, 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 *Tapirira guianensis* 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. MATERIALS AND METHODS

2.1 Chemicals and Equipment

To perform the tests here, solvents from J.T. Baker – Radnor, USA; Êxodo Cientifica – 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 10S UV-Vis Spectrophotometer – Thermo Scientific – Vantaa, Finland. The oil analysis was performed on the Shimadzu QP-2010 equipment. The *in silico* assays used the systems: AutoDockVina, Discovery Studio™ Pymol and UCSF Chimera™.

2.2 Material Collection and Extraction of the Essential oil

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 400 g of fresh leaves, through of the hydrodistillation technique in a Clevenger type dispenser.

2.2.1 Gas chromatography mass spectrometry (GCMS)

The oil analysis was performed on the Shimadzu QP-2010 equipament, 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 carrier gas (24.2 mL / min), with constant linear velocity; injector temperature of 250ºC (split mode 1: 100); detector temperature of 250ºC. In addition, the heating ramp was programmed, initially, from 35ºC to 180ºC, with an increase of 4ºC / min to 180ºC and, later, 17ºC / min to 280ºC, 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 through electronic impact with a 70eV energy beam. Consequently, mass spectra were generated, and the equipment suggested some compounds through comparison with an existing library. To effectively identify the oil components, the following parameters were analyzed: the chromatogram, mass spectra, Kovats indexes from the literature - based on the NIST and Adams database [13] - and the retention time of each compound. The experimental Kovat Index was calculated 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 ELX 800, with "Gen5 V2.04.11" software [14], with some modifications. For each well in the 96-well plates, the following solutions were used: 180 µL of a methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), and 20 µL of the extract sample dissolved in methanol, diluted 10 times to achieve a final concentration of 0.2 mg mL-1. Different oil concentrations were prepared, starting from an initial concentration solution of 2 mg mL-1, with subsequent dilutions to obtain concentrations of 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 over a total incubation time of 60 minutes. BHT was used as the standard for comparison, and 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 hours. Subsequently, 1 mL of this solution was added to 99 mL of ethanol. The absorbance was read at 734 nm (0.715). Various solutions of decreasing concentrations of Tapirira guianensis essential oil 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 [15]. The IC50 (mean inhibition concentration) was calculated using linear regression.

2.2.4 *In vitro* evaluation of acetylcholinesterase inhibition

The methodology described by ELLMAN et al. (1961) was utilized with some modifications to assess the inhibition of the essential oil against the enzyme acetylcholinesterase [16]. The potential for acetylcholinesterase inhibition was examined in 96-well flat-bottomed plates using a BioTek ELISA reader, model ELX 800, software "Gen5 V2.04.11." The reagents employed per well included: 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. 6H2O, 50 µL Tris/HCl solution with 0.1% bovine serum albumin, and 25 µL of samples/standards. Physostigmine (Eserine) and Galanthamine standards were assessed separately with 25 µL of AChE (0.22 unit, uL-1). The evaluation was performed in triplicate.

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

Species	Family	Part used	Exsiccate	Coordinators
*Tapirira guianensis*Aubl	Anacardiaceae	Leaves	64238	3°74'46.2"S 38°48'78.2"W

2.2.5 Determination of antifungal activity

Antifungal activity was assessed following 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 the Federal University of Pernambuco were utilized [17-18]. The MIC was determined in 96-well microplates, with the addition of 10 mg mL^{-1} of diluted extract, 50 µL of 5% DMSO, and 950 µL of RPMI medium. Additionally, 50 µL of RPMI medium was added to all wells in the first column, followed by a series of dilutions ranging from 0.002 to 2.5 mg mL^{-1} and 100 µL of the inoculum. The plates were incubated at 37 °C, and visual readings were conducted after 48 hours. Fluconazole was used as the positive control. The assays were performed in duplicate, and the MIC was defined as the lowest concentration of the 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 the solution removed from wells, without turbidity, on potato dextrose agar at 28 °C and defined 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 acetyleugenol (CID7136), α-copaene (CID19725), α-humulene (CID5281520), β-caryophyllene (CID5281515), Eugenol (CID3314), and the drug physostigmine (CID5983) were retrieved from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov/), and they were saved at physiological pH using the Marvin Sketch code [19]. The mechanisms of action of the 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 AutoDockVina code [21], configured to run the Lamarkian 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. ™ [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™ [62], Pymol [61] and UCSF Chimera™ [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 Autodocktools™ 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, 55 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.

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].

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
Acetyleugenol	1524	1523	7,52	-
Total			**100**	

Eugenol was the majority compound identified in the essential oil of *T. guianensis* leaves and is classified as a phenylpropranoid. It is considered the main phenolic compound in clove essential oil, so it is obtained from the shoots and leaves of *Eugenia caryophyllata* [64-65]. The constituent acetyleugenol is classified as a vanilloid. It exhibits antioxidant property that has already been reported. Diets rich in both eugenol and acetyleugenol 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 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 correlation 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 Cocó 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. cattapa*, with the content of total phenols in the range of 297.46 ± 26. 94 µg.mL^{-1} to 599.30 ± 17.08 µg.mL^{-1} with antioxidant activities with IC50 for the DPPH radical ranging from 3.44 ± 0.16 to 3.73 ± 0.12 µg.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).

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 (IC50 = 11.7 µg/mL), as well as its inhibition on reactive oxygen species (ROS) (IC50 = 1.6 µg/mL), H2O2 (IC50 = 22.6 µg/mL and 27.1 µg/mL) and NO (Nitric oxide) (IC50< 50.0 µg/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* Aubl 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. guianenis* 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 ± 0.14nM [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 constituents 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 evaluated *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, 0128, 0102 and 0104. The MIC values ranged from 156 to 312 μg/mL and MFC from 312 to 625 μg/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 μg/mL present strong activity; MICs between 500.0-1500.0 μg mL-1 promote moderate activity and MICs above 1500.0 μg/mL low activity. The MICs found for the strains in the present study show strong antifungal activity of *T. guianensis* essential oil.

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 1.932Å. 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).

Analyzing the interaction patterns against AChE (Table 6), it was possible to identify that the acetyleugenol/AChE complex is formed by two hydrophobic interactions, one with the *non-polar* side chain of the aromatic residue Trp 86ª (4. 12 Å) and one with the basic side chain residue His 447A(4.90 Å), two H-bondstrong interactions with the uncharged polar side chain residues Tyr 124A (2.45 Å), Ser 125A (2.12 Å) and a T-shaped Pi-Pi interaction with the non-polar side chain of the residue Trp 86A (5.06 Å). The α-Copaene/AChE complex is formed by eight hydrophobic interactions, six with the *non-polar* 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

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

	DPPH IC$_{50}$(µg/mL)	ABTS$^+$ IC$_{50}$(µg/mL)	ACHE IC$_{50}$(µg/mL)
Essential oil	4.39±0.076	5.24±0.023	12.56±0.012
BHT	1.61±0.04	0.95±0.06	-
Physo	-	-	1.15±0.05

BHT: butylated hydroxytoluene (Standard); Physo: physostigmine (Standard)

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

Samples	Strains	MIC(µg/mL)	MFC(µg/mL)
OE		156	312
	0131		
FLZ		1	1
OE		312	625
	0128		
FLZ		0.25	0.25
OE		156	312
	0102		
FLZ		0,25	0,25
OE		156	312
	0105		
FLZ		0.25	0.25

MIC: Minimum inhibitory concentration; MFC Minimum fungicidal concentration; FLZ: Fluconazole (Standard) and OE: Essential oil

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

Ligand	Affinity Energy (kcal/mol)	RMSD (Å)
Acetyleugenol	-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-cristalized (redocking)

the uncharged polar side chain residue Tyr 124(5.32 Å) and one with the basic side chain residue His 447A (4. The α- Humulene/AChE complex is formed by five hydrophobic interactions, two with the *non-polar* 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 *non-polar* 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 residueTyr 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 *non-polar* 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 *non-polar* 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 Ser 125(1.11 Å). Physostigmine/AChE is formed by five hydrophobic interactions, four with the *non-polar* 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 Tyr 124B. Therefore, the 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)
Acetyleugenol	Trp 86A	Hydrophobic	4.12
	His 447A	Hydrophobic	4.90
	Tyr 124A	H-Bond	2.45
	Ser 125A	H-Bond	2.12
	Trp 86A	Pi-Pi T-shaped	5.06
α-copaene	Trp 86A	Hydrophobic	3.72
	Trp 86A	Hydrophobic	3.95
	Trp 86A	Hydrophobic	4.17
	Trp 86A	Hydrophobic	4.44
	Trp 86A	Hydrophobic	4.86
	Tyr 124A	Hydrophobic	5.32
	Phe 338A	Hydrophobic	5.23
	His 447A	Hydrophobic	4.81
α -humulene	Trp 86B	Hydrophobic	3.86
	Trp 86B	Hydrophobic	4.10
	Tyr 124B	Hydrophobic	4.62
	Tyr 337B	Hydrophobic	4.80
	His 447B	Hydrophobic	5.40
β-cariophyllene	Trp 86B	Hydrophobic	4.63
	Trp 86B	Hydrophobic	5.28
	Tyr 337B	Hydrophobic	4.27
	Tyr 337B	Hydrophobic	4.75
	Phe 338B	Hydrophobic	4.93
	Phe 338B	Hydrophobic	5.44
	His 447B	Hydrophobic	4.65
Eugenol	Trp 86A	Hydrophobic	4.01
	Trp 86A	Hydrophobic	4.39
	Tyr 337A	Hydrophobic	3.61
	Tyr 449A	Hydrophobic	5.15
	Gly 120A	H-Bond	3.60
	Gly 121A	H-Bond	3.50
	Trp 86A	Pi-PiStacked	4.20
	Trp 86A	Pi-PiStacked	4.84
	Ser 125A	Unfavorable Donor-Donor	1.11
Physostigmine	Trp 86B	Hydrophobic	3.53
	Trp 86B	Hydrophobic	4.09
	Trp 86B	Hydrophobic	4.20
	Trp 86B	Hydrophobic	4.30
	Tyr 337B	Hydrophobic	4.24
	Tyr 124B	H-Bond	2.40
	Glu 202B	H-Bond	3.58
	Phe 338B	Pi-Pi T-shaped	5.78

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

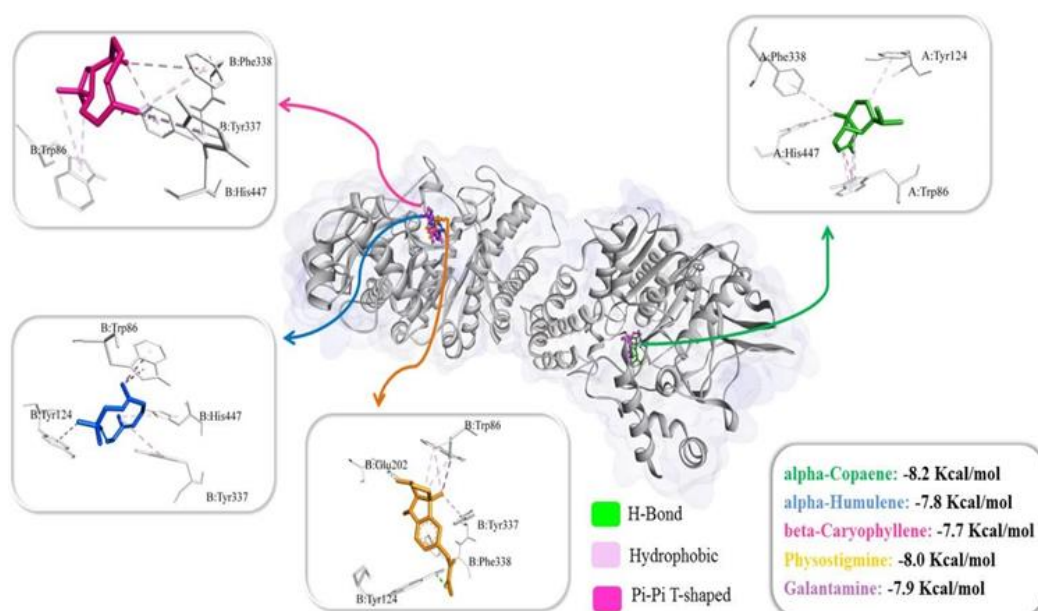| SAP5/Ligand	Energy (kcal/mol)	RMSD (Å)
Acetyleugenol	-5.8	1.268
α-copaene	-7.0	0.827
α-Humulene	-6.0	1.730
β-caryophyllene	-6.2	1.103
Eugenol	-5.7	1.777
Fluconazole	-7.2	1.676
PepA[*] - Ligand co-cristalized (redocking)	-8.0	1.635

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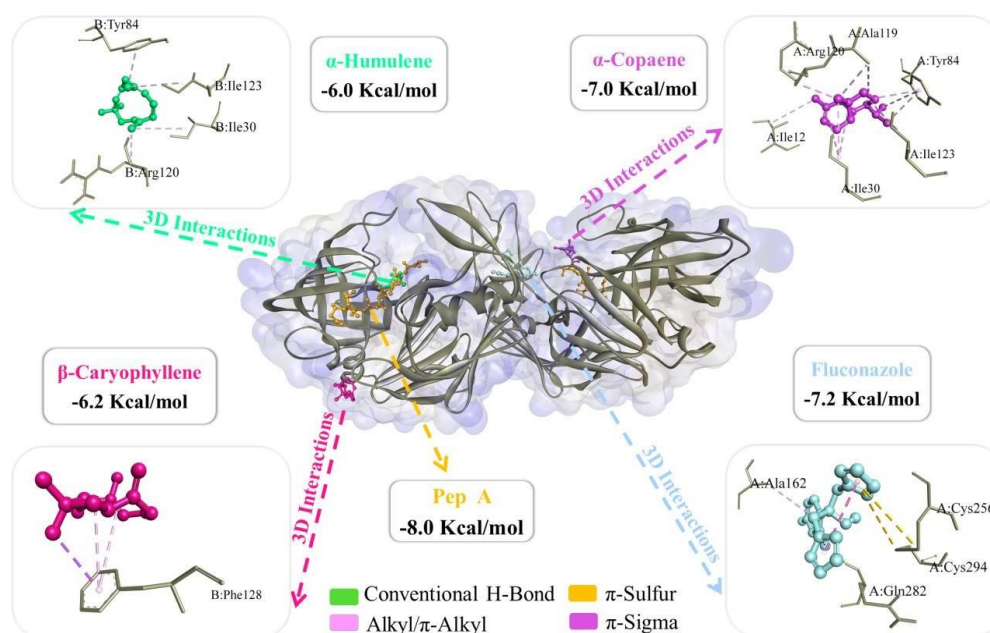

Fig. 2. Interaction complex between SAP5, α-Copaene, α-Humulene, β-caryophyllene, Fluconazole and the co-crystallized inhibitor Pepstatin A (PepA).

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 (acetyleugenol and α-copaene);Trp 86B, Tyr 337B and His 447B (α-humulene e beta-caryophyllene); Trp 86A, Gly 120A, Gly 121A, Tyr 337A (eugenol).

Compared to the drug physostigmine, we observed that acetyleugenol, 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 337Be Phe 338B (beta-caryophyllene), besides binding in the same region of the GNT site (Chain B), indicating that these compounds have similar action to the controls used. Fig. 1 shows the most stable receptor-ligand complexes (lowest affinity energy).

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].

Against SAP5 (Table 8), the SAP5/acetyleugenol complex showed hydrophobic interactions with residues Ile 30A, Ile 123A, Tyr 84A, two H-bonds with residues Gly 85A, Asp 86A and a Pi-PiStacked 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 residuePhe

128B. SAP5/Eugenol showed hydrophobic interactions with residues Ala 162A, Lys 257B, Phe 281AeH-bonds with residues Ser 277A, Glu 278A. SAP5/fluconazole showed a hydrophobic interaction with Ala 162A, an H-bond with Gln 282A and two Pi-Sulfur interactions with residues Cys 256A, Cys 294A.

Regarding SAP5, we observed that α-Copaene, α-Humulene and β-caryophyllene ligands formed the most stable receptor-ligand complexes (Fig. 2). The SAP5/ α-Copaene complex is formed by hydrophobic interactions with the *non-polar* side chain of residues Ile 12A, Ile 30A, Ala 119A, with the uncharged polar side chain of residue Tyr 84A, and with the basic side chain of residue Arg 120A. SAP5/ α-Humulene is formed by hydrophobic interactions with the *non-polar* side chain of residues Ile 30B, Ile 123B, the uncharged polar side chain of residue Tyr 84B, and the basic side chain of residue Arg 120B.SAP5/ β- caryophyllene is formed by hydrophobic interactions with the *non-polar* side chain of the Phe residue 128B.SAP5/ Fluconazole (control) is formed by a hydrophobic interaction with the *non-polar* 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 Ile 12, Asp 32, Gly 34, Ser 35, Lys 83, Tyr 84, Gly 85, Asp 86, Ile 123,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.

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

Ligands	Residue	Interaction	Distance (Å)
Acetyleugenol	Ile 30A	Hydrofobic	3.80
	Ile 123A	Hydrofobic	4.15
	Tyr 84A	Hydrofobic	2.71
	Gly 85A	H-Bond	2.36
	Asp 86A	H-Bond	2.63
	Tyr 84A	Pi-PiStacked	5.03

Ligands	Residue	Interaction	Distance (Å)
α-Copaene	Ile 12A	Hydrofobic	5.31
	Ile 30A	Hydrofobic	4.72
	Ile 30A	Hydrofobic	4.86
	Ile 30A	Hydrofobic	5.46
	Tyr 84A	Hydrofobic	4.39
	Tyr 84A	Hydrofobic	4.43
	Tyr 84A	Hydrofobic	5.13
	Ala 119A	Hydrofobic	4.12
	Ala 119A	Hydrofobic	5.28
	Arg 120A	Hydrofobic	3.79
	Ile 123A	Hydrofobic	5.35
α-Humulene	Ile 30B	Hydrofobic	5.25
	Tyr 84B	Hydrofobic	4.05
	Arg 120B	Hydrofobic	4.20
	Ile 123B	Hydrofobic	5.22
β-caryophyllene	Phe 128B	Hydrofobic	2.80
	Phe 128B	Hydrofobic	4.53
	Phe 128B	Hydrofobic	5.15
Eugenol	Ala 162A	Hydrofobic	4.83
	Lys 257B	Hydrofobic	4.39
	Phe281A	Hydrofobic	4.21
	Ser 277A	H-Bond	2.76
	Glu 278A	H-Bond	2.31
	Glu 278A	H-Bond	2.56
	Glu 278A	H-Bond	3.07
Fluconazole	Ala 162A	Hydrofobic	5.49
	Gln 282A	H-Bond	2.25
	Cys 256A	Pi-Sulfur	5.78
	Cys 294A	Pi-Sulfur	5.89

4. CONCLUSION

*T. guianensis l*eaves show very promising antioxidant, anticholinesterase and antifungal activities, according to *In vitro* analyzes. *In vitro* tests showed promising actions of *Tapirira guianensis* 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 beta-caryophyllene have similar or superior action to the drug physostigmine, and also suggest a possible synergistic effect of acetyleugenol eugenol 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 infections fungal infections caused by *Candida albicans*. However, this protein target was not able to demonstrate the activity of the known antifungal 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.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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