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ABSTRACT

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Keywords: essential oil; *tapirira guianensis*; antioxidant; antifungal; acetylcholinesterase.

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ABSTRACT

The species Tapirira guianensis Aubl is utilized in traditional medicine for treating leprosy, diarrhea, and syphilis. The present study aims to assess the chemical composition as well as the antioxidant, anticholinesterase, and antifungal potential of the essential oil extracted from the leaves of T. guianensis. The plant material was collected at Arco Estadual do Cocó, with prior authorization from the Municipal Environment Department. The essential oil was obtained through the hydrodistillation process using a Clevenger-type apparatus, and its constituents were analyzed via Gas Chromatography coupled to Mass Spectrometry. The antioxidant potential was determined by assessing free radicals, specifically DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS+ (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), using a 96-well plate. Antifungal activity was assessed following the protocol of the Clinical Laboratory Standards Institute - M38-A (CLSI, 2018). Gas chromatography coupled with mass spectrometry revealed the presence of constituents in T. guianensis essential oil, including eugenol (59.00%), α-copaene (0.40%), β-caryophyllene (29.91%), and α-humulene. The essential oil exhibited significant antioxidant and anticholinesterase potential in in vitro studies. This study represents the first exploration of the biological potential of T. guianensis leaf essential oil, highlighting its considerable potential for future investigations within the scientific community. Consequently, we can infer that T. guianensis essential oil serves as a source of antioxidant, anticholinesterase, and antifungal compounds, presenting promising therapeutic potential in the management of Alzheimer's disease and Candida infections.

Keywords: essential oil; *tapirira guianensis*; antioxidant; antifungal; acetylcholinesterase.

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I. INTRODUCTION

The species *Tapirira guianensis* Aubl belongs to the family Anacardiaceae. Species within the genus Tapirira are found from southern Mexico to South America (Roumy et al., 2009). Tapirira obtusa (Benth.) JD Mitch., T. pilosa Sprague, T. retusa Ducke, and T. guianensis Aubl. are examples of species from this genus that have been previously documented in Brazilian territory, commonly known as pau-pombo or pigeon chest. In traditional medicine, the leaves of *Tapirira guianensis* are widely used for treating leprosy, diarrhea, and syphilis (David et al., 1998).

Additionally, antioxidant and cholinesterase activities have been reported for both the leaves and stem bark of *Tapirira guianensis* (Oliveira et al., 2022).

Various approaches have been utilized in individuals with Alzheimer's disease, including the use of antioxidant substances and acetylcholinesterase inhibitors. Acetylcholinesterase inhibitors enhance the levels of the neurotransmitter acetylcholine, facilitating more efficient and prolonged nerve synapses within the cholinergic complex (Araújo; Santos; Gonsalves, 2016). Additionally, because free radicals contribute to the oxidation of biomolecules, resulting in the loss of their biological activities and/or disrupting homeostasis, the use of antioxidant substances represents a therapeutic strategy against neurodegenerative diseases (França et al., 2013; Duthie; Duthie; Kyle, 2000; Frota et al., 2022).

Most of the drugs used in patients with Alzheimer's disease (AD) do not offer a definitive treatment or cure, and their prices are typically high. The efficacy of these drugs often falls short, leading to a substantial demand within the industry for the exploration of new medications that exhibit greater effectiveness and minimal side effects.

Plants with high levels of phenolic compounds, such as Anacardium occidentale, Ceiba pentandra, Laguncularia racemosa, Mangifera indica, Myracrodrum urundeuva, and Terminalia catappa, demonstrated excellent antioxidant activity against the DPPH radical, with IC50 values ranging from 3.44 ± 0.16 to 3.73 ± 0.12 µg mL-1. Moreover, these plants exhibited a high power of inhibition of the AChE enzyme (IC50 < 20 µg mL-1). As a result, they are recommended for more specific studies related to Alzheimer's disease (De Morais et al., 2021).

In recent decades, microbial infections have been on the rise, leading to a substantial increase in morbidity and mortality rates. The resistance patterns exhibited by these microorganisms and the emergence of new pathogens have posed a significant challenge in the effort to eradicate infections. Global public health is adversely affected by the limited effectiveness of the antimicrobial drugs currently in use. Numerous studies have reported on the combat against pathogenic microorganisms using natural products derived from plants, animals, and microorganisms. Natural products have demonstrated considerable efficacy in treating infectious diseases, along with low-intensity side effects, synergy, and the potential to overcome drug resistance (Ye et al., 2020).

Essential oils are mixtures of volatile constituents, generally exhibiting terpenic or aryl-propanoid structures, which display diverse biological activities. The study of essential oils with antimicrobial activities is well-established (Swamy et al., 2016), as is their inhibition of the AChE enzyme. Commercially available essential oils extracted from Artemisia dracunculus L., Inula graveolens L., Lavandula officinalis Chaix, and Ocimum sanctum L., as well as the components of these oils, were screened using the microplate assay method to determine their acetylcholinesterase (AChE) inhibitory activity. The results indicated that the oils exhibited significant activity, and among the essential oil components, five compounds—namely, 1,8-cineole, α-pinene, eugenol, α-terpineol, and terpinen-4-ol—showed superior AChE inhibitory activity, with eugenol being particularly noteworthy (Dohi et al., 2009).

A practical method for comparing the activities of the constituents of essential oils can be achieved through a computational theoretical study of enzyme inhibition related to the studied diseases. For Alzheimer's disease, the enzyme AChE, and for fungal infections, the enzyme Als3 adducts. Based on docking and molecular dynamics studies of thymol and thymol acetate present in the essential oil of Lippia thymoides, these compounds were found to 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 can interact with the protein and inhibit its catalytic activity (Silva et al., 2019).

The present research aims to characterize the essential oil of Tapirira guianensis Aubl. leaves as a potential source of antioxidant, anti-acetylcholinesterase, and antifungal agents. These agents are considered valuable in addressing the symptoms associated with Alzheimer's Disease and Candidiasis. The study employs a combination of in vitro and in silico approaches to assess the properties of the essential oil.

Experimental

II. MATERIALS AND METHODS

2.1 Material collection

The plant material was gathered from a garden within Cocó State Park, Fortaleza, Ceará, Brazil. The exsiccates were identified by botanist Luiz Wilson Lima-Verde and subsequently deposited in the Prisco Bezerra Herbarium at the Federal University of Ceará (UFC). Authorization for the collection of plant material was obtained from the State Secretariat for the Environment of Ceará through permit 04/2021. The essential oil was extracted using approximately 400g of fresh leaves employing the hydrodistillation technique with a Clevenger-type apparatus.

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 Gas Chromatografy - Mass Spectrometry (GC/MS)

Oil analysis was conducted using the Shimadzu QP-2010 equipment under the following conditions: Rtx-5MS chromatographic column (Crossbond 5%, diphenyl/95% dimethyl-polysiloxane), a capillary column (30m x 0.25mm x 0.25 μm) coated with fused silica. Helium served as the carrier gas at a flow rate of 24.2 mL/min with constant linear velocity. The injector temperature was set at 250ºC in split mode 1:100, and the detector temperature was also maintained at 250ºC. The heating ramp was programmed to start at 35ºC and increase at a rate of 4ºC/min until reaching 180ºC. Subsequently, a ramp of 17ºC/min was applied until reaching 280ºC, where it remained for the final 10 minutes. This configuration generated a chromatogram correlating the relative retention time with the sample peaks.

The mass spectrum was obtained via electron impact with a 70eV energy beam. Consequently, mass spectra were generated, and the equipment suggested potential compounds by comparing them with an existing library. To accurately identify the components of the oil, the following analyses were conducted: examination of the chromatogram, scrutiny of the mass spectra, referencing the Kovats indexes from literature—according to the NIST and Adams database (2007)—and consideration of the retention time for each compound. The experimental Kovat Index was calculated using linear regression.

2.3 Determination of antioxidant activity by the DPPH Method

The antioxidant potential was assessed in 96-well flat-bottomed plates using a BioTek ELISA reader, model ELX 800, with "Gen5 V2.04.11" software (Becker et al., 2019), incorporating some modifications. In each well of the 96-well plates, the following solutions were utilized: 180 μL of a methanolic solution of DPPH (2,2-diphenyl-1-picrylhydrazyl), and 20 μL of the extract sample dissolved in methanol, diluted tenfold to achieve a final concentration of 0.2

mg/mL. Various oil concentrations were prepared from the initial solution with concentrations of 2 mg/mL, 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL, 3.12 μg/mL, 1.56 μg/mL, and 0.78 μg/mL. Absorbances were recorded at 490 nm over a 60-minute incubation period. The BHT (butylated hydroxytoluene) was used as the standard for comparison. All samples were analyzed in triplicate.

2.4 Assessment of Antioxidant Activity by the ABTS Method

The ABTS^+• solution (7 mM, 5 ml) was combined with 88 μl of potassium persulfate (140 mM). The mixture was stirred and kept in the dark at room temperature for 16 hours. Following this, 1 ml of this solution was added to 99 ml of ethanol. The absorbance was measured at 734 nm, resulting in a value of 0.715. Various solutions with decreasing concentrations of Tapirira guianensis essential oil were prepared. Subsequently, 3.0 ml of ABTS^+• solution was added to 30 μl of these oil solutions, and after 6 minutes, readings were taken at 734 nm (Re et al., 1999). The IC50 (mean inhibition concentration) was calculated using linear regression.

2.5 In vitro evaluation of acetylcholinesterase inhibition

The methodology outlined by ELLMAN et al. (1961) was employed with certain modifications to assess the inhibition of the essential oil against the enzyme acetylcholinesterase. The potential inhibition of acetylcholinesterase was examined in 96-well flat-bottomed plates using a BioTek ELISA reader, model ELX 800, with "Gen5 V2.04.11" software. The reagents used 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 patterns were separately evaluated with 25 μL of AChE (0.22 unit, uL-1). The assessment was conducted in triplicate.

2.6 Determination of antifungal activity

The antifungal activity was evaluated following the procedure outlined by Fontenelle et al. (2007) using broth microdilution Minimum Inhibitory Concentration (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. The MIC was determined in 96-well microplates by adding 10 mg mL-1 of diluted extract (50 μL of 5% DMSO and 950 μL of RPMI medium) and 50 μL of RPMI medium to all wells in the first column. This was followed by the addition of a series of dilutions (0.002 to 2.5 mg mL-1) and 100 μL of the inoculum. The plates were then incubated at 37 °C, and visual readings were conducted after 48 hours. Fluconazole was used as the positive control. The assays were carried out 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 through visualization as recommended by CLSI. The minimum fungicidal concentration (MFC) was determined by subculturing 100 μL of solution removed from wells without turbidity onto potato dextrose agar at 28 °C. The MFC was defined as the lowest concentration that resulte.

III. 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 is listed in Table 2, along with their experimental and literature retention indices. The oil exhibited a yield of 0.15%, consistent with the reported range of 0.13% to 0.24% in the literature by Zoghbi et al. (2014). Among the constituents identified in the present study, α-copaene, β-caryophyllene, and α-humulene were found to be common with the study mentioned.

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	0,4	1380
β-cariophyllene	1417	1423	29,91	1417
α-humulene	1452	1455	3,17	1452
acetyleugenol	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 (Adams, 2007).

Eugenol was identified as the predominant compound in the essential oil of *T. guianensis*. Eugenol is an aromatic compound belonging to the phenol group. It is commonly obtained from natural essential oils of plants from the Lamiaceae, Lauraceae, Myrtaceae and Myristicaceae families, and is the most important component of clove oil (Syzygium aromatum)(Ulanowska and Olas, 2021). The constituent acetyleugenol is classified as a vanilloid, known for its reported antioxidant properties. Diets rich in both eugenol and acetyleugenol have been associated with a potential reduction in the risks of diseases such as cancer, cardiovascular disorders, malaria, AIDS, and the effects of aging (Fujisawa et al., 2002; Satoh et al., 1998; Damiani, Rossoni & Vassallo, 2003).

The constituent α-copaene is classified as a tricyclic sesquiterpene, with a diverse range of applications in the food, drug, and agricultural industries (Zahin et al., 2021; Dong et al., 2020). The constituent β-caryophyllene was identified as the second major component of the essential oil from T. guianensis leaves. It is recognized as a sesquiterpene commonly found in essential oils of spices such as black pepper, cinnamon, and oregano, as well as in various plants, notably Cannabis sativa and Copaifera spp. (De La Cruz et al., 2013).

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 is derived (Katsiotis, Langezaal, & Scheffe, 1989). 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, with two of them being double substituted (Felipe and Bicas, 2017; Krivoruchko and Nielsen, 2015; Di Sotto et al., 2020)

3.2 Antioxidant and cholinesterase potential in vitro

The potential of antioxidant agents to neutralize DPPH radicals is commonly attributed to their hydrogen-donating ability. The reaction between DPPH and antioxidants is influenced by the structural conformation of the antioxidant compounds. Moreover, a higher number of hydroxyl groups in certain substances can lead to faster reactions against the DPPH radical (Bakari et al., 2015). Phenolic substances are well-documented in the literature as excellent antioxidant agents (Frota et al., 2022). The ortho-dihydroxylated (Melo et al., 2006; Frota et al., 2022) and para-dihydroxylated (Morais; Braz-Filho, 2007) positions in phenolic compounds contribute to a more pronounced antioxidant efficiency, although the exact mechanisms of action remain undefined.

Several authors have previously noted a correlation between antioxidant potential and phenolic compounds. A study involving 18 medicinal plants demonstrated that plants with higher total phenol levels exhibited more significant antioxidant effects in the DPPH (1,1-diphenyl-2-picrylhydrazyl) test (De Morais et al., 2013). Another study evaluated the relationship between phenolic compounds and antioxidant activity in 30 plants from Cocó State Park in Fortaleza, Ceará. A linear relationship was observed for 10 plants, including *A. occidentale*, *C. pentandra*, *H. stigonocarpa*, *L. racemosa*, *L. ferrea*, *M. indica*, *M. tenuiflora*, *M. urundeuva*, *S. mombim*, *T. cattapa*, with total phenol content ranging from 297.46 ± 26.94 µg/mL to 599.30 ± 17.08 µg/mL and antioxidant activities with IC50 for the DPPH radical ranging from 3.44 ± 0.16 to 3.73 ± 0.12 µg/mL, respectively (De Morais et al., 2021). Analyzing the antioxidant potential of the essential oil from the leaves of T. guianensis, a very promising action against the two radicals tested can be observed (Table 3).

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)

Eugenol, comprising the majority constituent at 59.00%, is likely to exert a significant influence on the antioxidant action. The capacity of eugenol to scavenge free radicals has been previously demonstrated in the DPPH assay (IC50 = 11.7 µg/mL), as well as its inhibition of reactive oxygen species (ROS) (IC50 = 1.6 µg/mL), H2O2 (IC50 = 22.6 µg/mL and 27.1 µg/mL), and NO (IC50 < 50.0 µg/mL) (Perez-Roses et al., 2016). This justifies the potential of T. guianensis essential oil attributed to the eugenol content in its composition. Although eugenol is the major constituent of *T. guianensis* Aubl essential oil and exerts a significant influence on the ability to eliminate free radicals, the constituents α-copaene, β-caryophyllene, and α-humulene also contribute to the biological action, as their antioxidant activities have been confirmed by other researchers.

Regarding the anticholinesterase action (Table 3), the essential oil from *T. guianensis* leaves also demonstrated a highly promising effect. Alzheimer's disease is characterized as a neurodegenerative pathology that affects the thinking, memory, learning, and behavior of affected individuals. Acetylcholinesterase is an enzyme that plays a role in terminating cholinergic signaling through the hydrolysis of acetylcholine. Therefore, inhibiting the acetylcholinesterase enzyme may represent a highly promising strategy in the treatment and management of the disease (Ozgeris et al., 2016; Gocer et al., 2016).

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 (Topal et al., 2017).

In some neurodegenerative diseases, the use of β-caryophyllene has been demonstrated to prevent neuronal death in models of focal ischemia (Chang et al., 2013), vascular dementia (Lou et al., 2017), Parkinson's disease (Cheng; Dong & Liu, 2014; Wang, Ma & Du, 2018), and Alzheimer's disease (Viveros-Paredes et al., 2017; Javed et al., 2016). It can be suggested that the constituents eugenol and β-caryophyllene influence the anticholinesterase action, given that there are already records regarding their cholinesterase effects. "

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 effect against Candida albicans. The test evaluated the oil's impact on four strains of Candida albicans: 0131 (clinical), 0128 (clinical), 0102 (clinical), and 0104 (clinical). The MIC values ranged from 156 to 312 µg/mL, and MFC ranged from 312 to 625 µg/mL (Table 4). Sartoratto and collaborators (2004) classified the antifungal activity of aromatic plants used in Brazil. According to their classification, FCMs lower than 500.0 µg/mL exhibit strong activity, MICs between 500.0-1500.0 µg/mL demonstrate moderate activity, and MICs above 1500.0 µg/mL indicate low activity. The MICs found for the strains in the present study reveal the 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(µg/mL)	MFC(µg/mL)
OE	0131 (clinical)	156	312
FLZ		1	1
OE	0128(clinical)	312	625
FLZ		0.25	0.25
OE	0102(clinical)	156	312
FLZ		0,25	0,25
OE	0105(clinical)	156	312
FLZ		0.25	0.25

MIC: Minimum Inhibitory concentration; MFC Minimum fungicidal concentration; FLZ: Fluconazole (Standard) and OE: essential oil

The antifungal action of eugenol against Candida albicans has already been evaluated in in vitro models. Its activity may be related to the alteration of the cell membrane and cell wall structure, leading to the release of cell contents (Bennis et al., 2004). Some authors state that the antifungal action may be related to plasma membrane instability, including denaturation of cytoplasmic proteins, with the ability to inactivate enzymes, causing cell death (Raut & Karuppayil, 2014). β-caryophyllene, when tested alone, already shows inhibitory action on fungal development (Fernandes et al., 2007). 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.

IV. CONCLUSIONS

The results of in vitro tests revealed promising activities of the essential oil of *Tapirira* *guianensis* in the areas of antioxidant, cholinesterase, and antifungal activities. The substances eugenol and β-caryophyllene, already well-studied in the literature due to their broad pharmacological spectrum, seem to positively influence the biological activities assessed in this study, highlighting the potential of the essential oil as a natural source of bioactive substances. In this context, the essential oil of Tapirira guianensis emerges as a possible tool in therapeutic strategies against Alzheimer's disease. Furthermore, the oil demonstrated excellent efficacy against Candida infections, positioning it as a strong candidate for the production of formulations to combat these infections. Further investigations into the specific constituents of the oil are recommended to understand the underlying mechanisms of action for these biological activities. Additionally, in vivo assays and preclinical studies with the identified substances or formulations containing these active principles can be conducted to contribute to the development of effective pharmaceutical prototypes.

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