Original Research Article

CG-MS, radical scavenging, acetylcholinesterase inhibition, antifungal and molecular docking studies of essential oil from the leaves of *Tapirira guianensis* Aubl

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.

Keywords: Essentialoil;Tapirira guianensis;Antioxidant;Antifungal;Acetylcholinesterase.

1. INTRODUCTION

The *Tapirira guanensis*Aubl belongstothefamilyAnacardiaceae.Species of the genus*Tapirira*rangefrom southern MexicotoSouth America [1].*Tapiriraobtusa*(Benth.)JDMitch.,*T.pilosa*Sprague,*T.retusa*Duckeand *T.guianensis*Aubl.,arespeciesofthegenusthathavealreadybeenreportedhere in Brazilian territory, where itiscommonly known as pau-pombo or pigeon chest. In folkmedicine, its leaves are widely used to treat leprosy, diarrhea and syphilis [2].Antioxidantandcholinesteraseactivitieshavealsobeenreportedfortheleavesandstembarkof*Tapirira guianensis*[3].

Some approacheshavebeen usedinindividualswithAlzheimer's disease suchastheuseofantioxidantsubstancesand acetylcholinesterase

inhibitors.Acetylcholinesteraseinhibitorspromoteincreasedlevelsoftheneurotransmitteracetylcholine,enablingmoreefficient andlong-lastingnervesynapseswithinthecholinergic complex [4]. Moreover, becausefree radicalsparticipateintheoxidationofbiomoleculesandleadtothelossoftheirbiologicalactivitiesand/orhomeostaticimbalance,t heuseofantioxidantsubstancesisalsoconfiguredas atherapeuticstrategy againstneurodegenerativediseases [5-6-7].

Mostof thedrugsusedinpatientswithAD(Alzheimer's Disease)donotprovideatreatmentand/orcure,andthepricesofthesedrugsarehigh.Thereisalackofpositiveoutcomesinpatients whouse the drugs,bringing with itahigh demandfortheindustry,with respecttothesearchfornewdrugswithgreatereffectivenessandlowsideeffects.

Plants with high levels of phenolic compounds such as*Anacardium occidentale*,*Ceibapentandra*,*Laguncularia racemosa*,*Mangiferaindica*,*Myracrodrumurundeuva*and*Terminaliacatappa*sho wedexcellentantioxidantactivity againstthe DPPHradical(withIC50rangingfrom3.44±0.16to3.73±0.12µgmL^{-1})andhighpowerofinhibition of the AChE enzyme (IC50< 20 µg mL^{-1}) were recommended for more specificstudiesrelatedtoAlzheimer'sdisease[8].

Inrecentdecades,microbialinfectionshavebeenontherise,andwithit,morbidityandmortalityrateshavealsoincreasedsignifica ntly.Boththeresistancepatterns of these microorganisms and the proliferation of new pathogens have become achallengeinthe eradication of infections.Publichealth worldwide suffersbecause of theloweffectivenessoftheantimicrobialdrugsthatareused.Severalstudieshavebeenreportedonthefightagainstpathogenicmi croorganismsusingnaturalproductsfromplants,animalsandmicroorganisms.Naturalproductshaveshownsignificantefficacyf orthetreatmentofinfectiousdiseases,aswellasdemonstratinglow-intensitysideeffects,synergyandthepotentialtoovercomedrugresistance[9].

Essential oils are of volatile constituents generally of terpenic or aryl propanoid structures that exhibit varied biological activities. The study of essential oil withantimicrobialactivitiesarewellknownaswellasAChEenzymeinhibition[10].Commerciallyavailableessentialoilsextractedf rom*Artemisiadracunculus*L.,*Inulagraveolens*L.,*Lavandulaofficinalis*Chaix,and*Ocimumsanctum* L.andthecomponentsoftheseoilswerescreenedbymicroplateassaymethodtodeterminetheiracetylcholinesterase(AChE)inh ibitory activity.Theresultsshowedthatthe oils, with exposed activity and among the essential oil components, five components,namely1,8-cineole,α-pinene,eugenol,α-terpineolandterpinen-4-ol,showedbetterAChEinhibitoryactivity,highlightingtheconstituenteugenol[11].

Apracticalwaytocomparetheactivitiesoftheoils'constituentscanbedonethroughacomputationaltheoreticalstudyoftheinhibitio nofenzymesrelatedtothestudieddiseases.ForAlzheimer'sdiseasetheenzymeAChEandforfungalinfectionstheenzymeAlS3a dducts.Basedonthedockingandmoleculardynamicsstudiesofthymolandthymolacetatepresentintheessentialoilof*Lippiathy moides*,thesecompoundsinteractwith thecatalyticresiduesSer203andHis447of theactivesiteofacetylcholinesterase.Thefree bindingenergies(ΔG bind)for theseligands were-18.49and-26.88 kcal/mol,demonstratingthattheligands are able tointeractwith the proteinandinhibititscatalyticactivity[63].

Thepresentresearchaimstoqualifytheessentialoiloftheleavesof*Tapirira guianensis*Aubl.asasourceofantioxidant,anti-acetylcholinesteraseandantifungalagents,usefulincombatingthesymptomsofAlzheimer'sdiseaseandcandidiasis,through*in vitro*and*in silico*studies.

2. MATERIAL AND METHODS

2.1. ChemicalsandEquipment

To perform the tests here, solvents from J.T. Baker – Radnor, USA; ÊxodoCientifica –SãoPaulo,BR;andNeon– SãoPaulo,Brazil.ReagentswerepurchasedfromSigma-Aldrich- St. Louis, USA and Carvalhães – Alvorada, RS, Brazil. The devices used were a BiotekELISAreader–Vermont,USA;Genesys10SUV-VisSpectrophotometer–Thermo Scientific – Vantaa, Finland. The oil analysis was performed on the Shimadzu QP-2010equipment. The *in silico* assays used the systems: AutoDockVina, Discovery Studio™ PymolandUCSFChimera™.

2.2. Materialcollection and Extraction of the essential oil

The plant material was collected from a garden of the Cocó State Park, Fortaleza,Ceará,Brazil.Exsiccateswereidentifiedbythebotanist LuizWilsonLima-Verdeanddeposited in the PriscoBezerra Herbarium of the Federal University of Ceará (UFC). Thelicenseforthecollectionof plantmaterial wasgrantedby theState

SecretariatfortheEnvironment of Ceará through authorization 04/2021. The essential oil was extracted usingabout 400g of fresh leaves, through of the hydrodistillation technique in a Clevenger typedispenser.

Table1.Identification of thespecies collectedin theCocóState Park in Fortaleza,Ceará,Brazil

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

2.2.1. GasChromatographyMassSpectrometry(GCMS)

The oil analysis was performed on the Shimadzu QP-2010 equipament, under thefollowingconditions:Rxt-5MSchromatographic column(Crossbond5%,diphenyl/95%dimethylpolysiloxane),capillary(30mx0.25mmx0.25µm)coatedwithfusedsilica;Helium ascarriergas(24.2mL/min),withconstantlinearvelocity;injectortemperatureof250ºC(splitmode1:100);detectortemperatureof 250ºC.Inaddition,theheatingrampwasprogrammed,initially,from 35ºCto180ºC,withanincreaseof4ºC /min to180ºC and,later,17ºC /min to280ºC,remaining atthat temperatureforthefinal10minutes.Thus,thechromatogramwasgenerated,whichrelatestherelativeretentiontimetothesample 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 activitybythe DPPHMethod

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 AntioxidantActivitybytheABTSMethod

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*evaluationofacetylcholinesterase 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..

2.2.5. Determination ofantifungalactivity

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. Moleculardocking - In silicoevaluationofacetylcholinesteraseinhibition andanti-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 64algorithm[22].Tovalidatethesimulationsperformed,theredockingtechnique was performed with the drug Galantamine (GNT) co-crystallized in the AChEtarget. ™ [23]. To validate the coupling simulations in the evaluationagainst *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 validatethe docking simulations, the redocking technique was performed with the co-crystallizedinhibitor PepA (SAP5). [24]. Residues were removed, polar hydrogens added andGasteigerchargescalculated[25]usingtheAutodocktools™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 statisticalparameter 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 usedasacriteriontoselectthe bestpose.

The strength of the hydrogen bonds (H bond) was analyzed through the values of thedistances between the donor and acceptor atoms, classified as Strong bonds when they presentdistances between 2.5-3.1 Å, Medium bonds between 3.1-3, 55 Å and Weak bonds when theyhave adistancegreaterthan3.55Å[32].

3. RESULTS AND DISCUSSION

3.1. CHEMICALCONSTITUTIONOFTHEESSENTIALOILOFTAPIRIRA GUIANENSIS

The percentage of compounds identifiedin the essential oil from theleaves arelistedin Table 2 along with their experimental and literature retention indices. The oil showed ayield 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 andα-humulene werecommonwiththe studyfound.

Table2.Relativepercentagecompositionoftheessentialoilof*T.guianensis*leavesbygaschromatography-massspectrometry(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	

Kovatsindexes(KI)wereestimatedbylinearregressionofretentiontimesofmaincompounds in the chromatograms and respective Kovats index from the literature [13].

Eugenolwasthemajoritycompoundidentifiedintheessentialoilof*T.guianensis*leavesandisclassifiedasaphenylpropranoid.Itis consideredthemainphenolic compound in clove essential oil, so it is obtained from the shoots and leaves of*Eugeniacaryophyllata*[64-65].Theconstituentacetyleugenolisclassifiedasavanilloid.Itexhibitsantioxidantpropertythathasalreadybeenreported.Dietsrichinbotheugenolandacetyleugenolmayreducetherisks of diseasessuchascancer,cardiovasculardisorders,malaria,AIDS,andtheeffectsofaging[34-35-36].

Theconstituentα-copaeneisclassifiedasatricyclicsesquiterpene.Ithasarangeofapplicationsacrossthefood,drug,andagriculturalindustries [37-66].Theconstituentβ-caryophyllenewas thesecondmajor constituentofthe essential oilfrom *T.guianensis*leaves.Itis consideredasesquiterpene widelyfoundinessentialoilsofspicessuchasblackpepper,cinnamonandoreganoandalsoinvariousplants,mainly*Cannabissativa*and*Copaifera*spp.[38].

Theconstituentα-humuleneisclassifiedasanaturallyoccurringmonocyclicsesquiterpene.Itisoneof theconstituentsof theessentialoilofthefloweringconeofthehopplant,*Humuluslupulus*,fromwhichitsnamecomes[39].α-Humuleneorα-Caryophyllene((1E,4E,8E)-2,6,6,9-tetramethylcycloundeca-1,4,8-triene)containsinitsstructureaneleven-memberedringcontainingthreetrans-endocyclic(1-2,4-5and8-9)doublebonds,wheretwoaredoublesubstituted[40-41-42].

3.2. Antioxidantandcholinesterasepotential in vitro

ThepotentialofantioxidantagentstoeliminateDPPHradicalsiscommonlyattributed totheirhydrogen-donating ability. The reaction of DPPH with antioxidants isinfluenced by the structural conformation of the antioxidant compounds. In addition, theamountof hydroxylsin somesubstances can provide faster reactions againstthe DPPHradical [67].Phenolicsubstancesarealreadywelldescribedintheliteratureasexcellentantioxidantagents[43].Theortho-dihydroxylated[44-45]andpara-dihydroxylated[46]positionsinphenoliccompoundsprovideamorepronouncedantioxidantefficiency,eventhoughthemechanismsofactionarenotwelldefined.

Thereisacorrelationalreadymentionedbyseveralauthorsinrelationtotheantioxidant potential and phenolic compounds. A study with 18 medicinal plants showed thatplants with higher levels of total phenols promoted better antioxidant effects by the DPPH(1,1-diphenyl-2-picrylhydrazyl)test[47].Anotherstudyevaluatedtherelationship of phenolic compounds and antioxidantactivity of 30 plants from Cocó StateParkinFortaleza,Ceará.Alinearrelationshipwasobservedfor10plants:*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. 94μg.mL^{-1} to 599.30 ± 17.08 μg.mL^{-1} with antioxidant activities with IC_{50} for the DPPH radicalrangingfrom3.44±0.16to3.73±0.12μg.mL^{-1},respectively[8].Analyzing the antioxidant potential of the essential oil from the leaves of *T. guianensis*, onecannotice a verypromisingactionagainstthe tworadicalstested(Table 3).

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

	DPPHIC$_{50}$(μg/mL)	**ABTS^{+}IC$_{50}$(μg/mL)**	**ACHEIC$_{50}$(μg/mL)**
Essentialoil	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:butylatedhydroxytoluene(Standard);Physo:physostigmine(Standard)

Eugenol,whichispresentedasthemajorityconstituent(59.00%), shouldpossiblyhaveastronginfluenceontheantioxidantaction.Theabilityofeugenoltosequesterfreeradicalshasalreadybeendescribedintheassay(IC50=11.7µg/mL),as wellas its inhibition on reactiveoxygen species (ROS) (IC_{50}= 1.6 µg/mL),H_2O_2 (IC_{50}= 22.6µg/mL and 27.1µg/mL)andNO (Nitric oxide) (IC_{50}< 50.0µg/mL)[48]. With this, we can justify the potential of *T. guianensis* essential oil from theeugenolcontentinthecomposition.Althougheugenolisthemajorconstituentof*T.guianensis* Aublessential oil and it has a high influence on the ability to eliminate freeradicals,theconstituentsα-copaene,β-caryophylleneandα-humulenealsoparticipateinthe biological action, since their antioxidant activities have already been proven by otherauthors.

Regardingtheanticholinesteraseaction(Table3),theessentialoilfrom*T.guianenis*leavesalsoshowedaverypromisingaction.Alzheimer'sdiseaseischaracterizedasaneurodegenerativepathologythataffectsthinking,memory,learning,andbehavior of affectedindividuals.Acetylcholinesterase is an enzymethatacts in theterminationof cholinergicsignalingbyhydrolysisof acetylcholine.Withthis,inhibitionoftheacetylcholinesteraseenzymemaybeaverypromisingstrategyinthetreatmentandmanagementofthedisease [49-50].

UsingEllman'sspectrophotometricmethod,theconstituenteugenolandfivederivatives: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-methoxyphenoland3-(2-hydroxy-3-(4-hydroxy-3-methoxyphenyl)propoxy)naphthalen-2-olwereanalyzedfortheiractionininhibitingtheenzymeacetylcholinesterase.Thestudyshowedthatallcompoundsshowedpromisingactionagainsttheenzymewith$K_I$valuesrangingfrom 90.10±0.01-379.57±0.14nM[51].

Insomeneurodegenerativediseases,theuseofβ-caryophylleneacts inpreventingneuronaldeathinmodelsoffocalischemia[52],vasculardementia[53],Parkinson'sdisease [52-54]andAlzheimer'sdisease [55-56].Wecansuggestthattheconstituentseugenolandβ-caryophylleneinfluencetheanticholinesteraseactionastherearealreadyrecordsconcerningtheircholinesteraseeffect.Alltheconstituentsrecordedintheessentialoilof*T.guianensis*leaveswereevaluated*in silico*againsttheacetylcholinesteraseenzymeandwillbedemonstratedinthetopic(*In silico*evaluationofacetylcholinesteraseenzymeinhibition).

3.3. Determination ofantifungalactivity

Through*in vitro*analysisitwaspossibletodemonstratethattheessentialoilfrom *T.guianensis*leaves has an action against*Candida albicans*. The testevaluated theeffectoftheoilagainstfourstrainsof*Candidaalbicans*:0131,0128,0102and 0104. TheMICvalues rangedfrom 156 to312 µg/mL andMFCfrom312to625µg/mL(Table4).Sartorattoandcollaborators(2004)classifiedtheantifungalactivityofaromaticplantsusedinBrazil [57].Accordingtotheclassification:FCMslowerthan500.0µg/mL presentstrongactivity;MICsbetween 500.0-1500.0µgmL-1promotemoderateactivityandMICsabove1500.0µg/mLlowactivity.TheMICsfoundforthestrainsinthepresentstudyshowstrongantifungalactivityof*T.guianensis*essentialoil.

Table4.Anti-*Candida*potentialofthe essentialoilfromtheleavesof*T.guianensis*

Samples	**Strains**	**MIC(µg/mL)**	**MFC(µg/mL)**
OE	0131	156	312
FLZ		1	1

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

MIC:Minimuminhibitoryconcentration;MFCMinimumfungicidalconcentration;FLZ:Fluconazole(Standard)andOE:Essentialoil

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 wallstructure, leading to the release of the cell contents [58]. Some authors statethat the antifungal action may be related to plasma membrane instability, even denaturation ofcytoplasmic proteins,with the ability toinactivate enzymes,causingcell death [59].β-caryophyllene tested alone, already shows inhibitory action on fungaldevelopment [60]. Through the mentioned studies on eugenol and β-caryophyllene, we can justify that the antifungal potential of *T. guianensis* essential oil is duetothepresenceandcontents oftheseconstituents.

3.4. Moleculardocking-evaluationofin silicoresults - In silico evaluationofacetylcholinesteraseenzymeinhibition

TheobservedRMSDvaluespresentedvariationsfrom0.994to1.932Å.Withrespectto affinity energy,the receptor-ligand complexesformed exhibitedvaluesin therangeof-6.2to-8.2kcal/mol(Table5).

Table5. RMSDandaffinityenergyvaluescalculatedinmoleculardockingsimulations

Ligand	**AffinityEnergy(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

*Ligandco-cristalized(redocking)

Analyzing the interaction patterns against AChE (Table 6), it was possible to identifythattheacetyleugenol/AChEcomplexisformedbytwohydrophobicinteractions,onewiththe *non-polar* side chain of

the aromatic residue Trp 86ª (4. 12 Å) and one with the basic sidechain residue His 447A(4.90 Å), two H-bondstrong interactions with the uncharged polar sidechainresiduesTyr124A(2.45Å),Ser125A(2.12Å)andaT-shapedPi-Piinteractionwiththe non-polar side chain of the residue Trp 86A (5.06 Å). The α-Copaene/AChE complex isformed 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 the uncharged polar sidechain residue Tyr 124(5.32 Å) and one with the basic side chain residue His 447A (4. The α-Humulene/AChEcomplex isformedby fivehydrophobicinteractions,two with the *non-polar*side chain residue Trp 86B (3.86 and 4.10 Å), two with the uncharged polar side chainresidues Tyr 124B (4.62 Å), Tyr 337B (4.80 Å) and one with the basic side chain residue His447B (5.40 Å). The beta-Caryophyllene/AChE complex is formed by seven hydrophobicinteractions, four with the *non-polar* side chain residues Trp 86B (4.63 and 5.28 Å), Phe 338B(4.93and5.44Å),twowiththeunchargedpolarsidechainresidueTyr337B(4.27and4.75Å) and one with the basic side chain residue His 447B (4.65 Å).Eugenol/AChE is formed byfour hydrophobic interactions, two with the *non-polar* side chain residue Trp 86A (4.01 and 4. 39Å), two with the uncharged polar side chain residues Tyr 337A (3.61 Å), Tyr 449A (5.15 Å),one H-bondaverage with the uncharged polar side chain residue Gly 121A(3.50 Å), one H-bondweak with the uncharged polar side chain residue Gly 120A (3.60 Å), two Pi-Pi Stackedinteractions with the *non-polar* side chain of the aromatic residue Trp 86(4.20 and 4.84 Å) and anUnfavorableDonor-Donorinteractionwiththeunchargedpolarsidechainresidue Ser125(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 polarside chain residue Tyr 337B (4.24 Å), one H-bondstrong with the uncharged polar side chainresidueTyr124B.Therefore,thesesquiterpenesα-copaene,α-humulene,andbeta-caryophyllene were shown to be the most active against the AChE enzyme by having the bestaffinityenergies.

Table6.Typesofinteractionsanddistances(Å)betweenligandsandaminoacidresiduesofAChE.

Ligands	Residue	Interaction	Distance(Å)
Acetyleugenol	Trp86A	Hydrophobic	4.12
	His 447A	Hydrophobic	4.90
	Tyr124A	H-Bond	2.45
	Ser125A	H-Bond	2.12
	Trp86A	Pi-PiT-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

ThebindingsiteofGalantamine(GNT)co-crystallizedbetweentheAandBchains of theAChEreceptorisformedby residues Trp 86,Gly 120,Gly 121,Gly 122,Glu202,Ser203,Phe295,Phe297,Tyr337andHis447[24].Theevaluatedcompoundscomplexinthesameregionof theGalantaminebindingsite,having in common interactions with residues Trp 86A and His 447A (acetyleugenol andα-copaene);Trp86B,Tyr337BandHis447B(α-humuleneebeta-caryophyllene);Trp86A,Gly120A,Gly121A,Tyr337A(eugenol).

Comparedtothe drugphysostigmine,we observedthatacetyleugenol,α-copaeneandeugenoldonotcompeteforthephysostigminebindingsite,suggestingapossiblesynergisticeffectwithphysostigmineandsimilaractiontoGNT(ChainA).Wealsoobservedthatα-humuleneandβ-caryophyllenecompoundscomplexinthesameregionofthephysostigminebindingsite,havingincommoninteractionswithresiduesTrp86B,Tyr124BandTyr337B(α-humulene),Trp86B,Tyr337BePhe338B(beta-caryophyllene),besides bindingin thesame region of the GNT site (ChainB),indicatingthatthese compoundshave similar action to the controls used.Figure 1 shows themoststablereceptor-ligandcomplexes(lowestaffinityenergy).

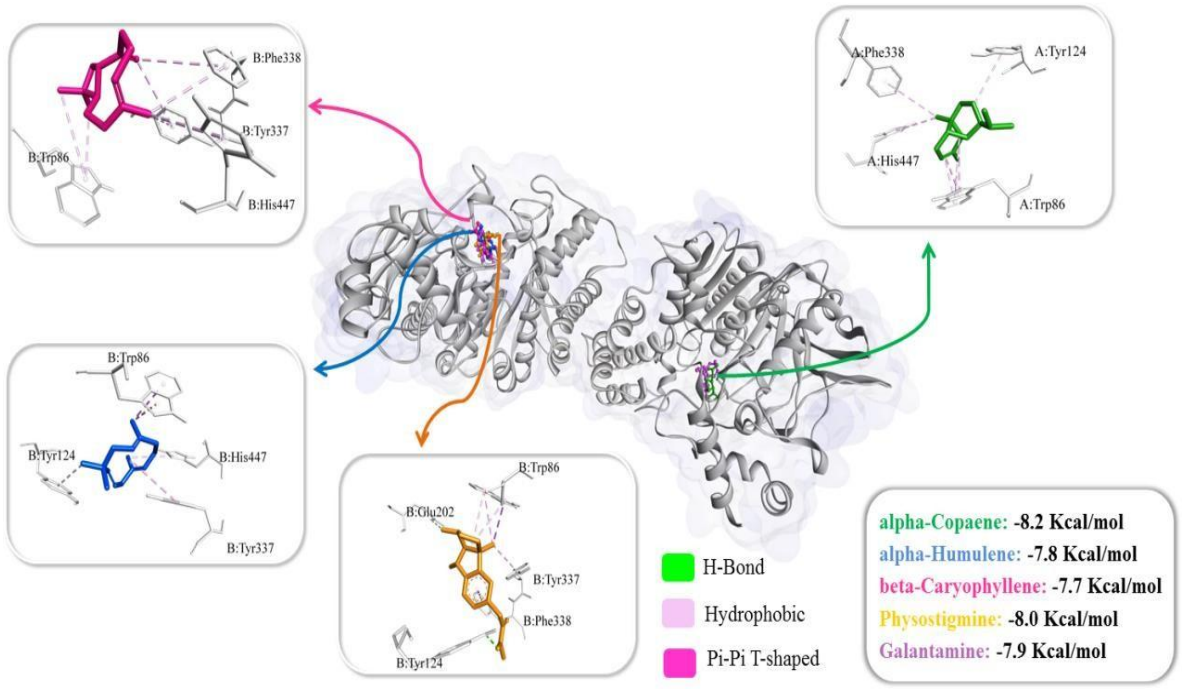

Fig.1.ComplexinteractionbetweenAChE,α-copaene(green),α-humulene(blue),β-caryophyllene (pink), physostigmine(orange) andthe co-crystallized inhibitor galantamine(purple).

3.5. In silicoevaluationoftheanti-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) andβ-caryophyllene (-6.2 kcal/mol) ligands presented values within the ideal range for energy ofaffinity (Table 7). This enzyme was not suitable for a more general evaluation of the activityagainst fungi since it did not find the well known antifungal action of eugenol [10].

Table7.AffinityenergyvaluesandRMSDofcomplexesformedwith*Candidaalbicans*

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*-Ligandco-cristalized(redocking)	-8.0	1.635

AgainstSAP5(Table8),theSAP5/acetyleugenolcomplexshowedhydrophobicinteractions 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 hydrophobicinteractions 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, Arg120BandIle123B.TheSAP5/β-

caryophyllenecomplexshowedthreehydrophobicinteractions with the residuePhe 128B. SAP5/Eugenol showed hydrophobic interactions withresiduesAla162A,Lys257B,Phe281AeH-bondswithresiduesSer277A,Glu278A.SAP5/fluconazole showed a hydrophobic interaction with Ala 162A, an H-bond with Gln282AandtwoPi-SulfurinteractionswithresiduesCys256A,Cys294A.

Table8.Typesofinteractionsanddistances(Å)betweenligandsandaminoacidresiduesofSAP5*Candidaalbicans.*

Ligands	Residue	Interaction	Distance(Å)
Acetyleugenol	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-PiStacked	5.03
α-Copaene	Ile12A	Hydrofobic	5.31
	Ile30A	Hydrofobic	4.72
	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
α-Humulene	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	Hydrofobic	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	Hydrofobic	5.49
	Gln282A	H-Bond	2.25
	Cys256A	Pi-Sulfur	5.78
	Cys294A	Pi-Sulfur	5.89

RegardingSAP5,weobservedthatα-Copaene,α-Humuleneandβ-caryophylleneligands formed the most stable receptor-ligand complexes (Figure 2). The SAP5/ α-Copaenecomplex is formed by hydrophobic interactions with the *non-polar* side chain of residues Ile 12A,Ile30A,Ala119A,withtheunchargedpolarsidechainofresidueTyr84A,andwiththebasicsidechainofresidueArg120A.SAP 5/α-Humuleneisformedbyhydrophobicinteractionswiththe*non-polar*sidechainof residuesIle30B,Ile123B,theunchargedpolarsidechainofresidueTyr84B,andthebasicsidechainofresidueArg120B.SAP5/β-caryophyllene is formed by hydrophobic interactions with the *non-polar* side chain of the Pheresidue 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 chainresidue charged Gln 282A (2.25 Å) and two Pi-Sulfur interactions with the uncharged polarside chain residues Cys 256A and Cys 294A. The active site of the Sap5/PepA complex isformedbyresiduesIle12,Asp32,Gly34,Ser35,Lys83,Tyr84,Gly85,Asp86,Ile123,Gly 220, Thr 221, Thr 222, Ile 223 and Ile 305 [24]. Interaction analysis showedthat α-copaene and α-humulene interact with amino acid residues of the active site of theSap5/PepA complex (α-Copaene, Ile 12A, Tyr 84A and Ile 123A; α-Humulene, Tyr 84B andIle 123B), which indicates a similar action to the PepA inhibitor and the possibility of asynergistic effect with Fluconazole. β-caryophyllene interacts at a different binding site thanPepstatin A and the control, which indicates a possible synergistic effect with PepA andFluconazole.

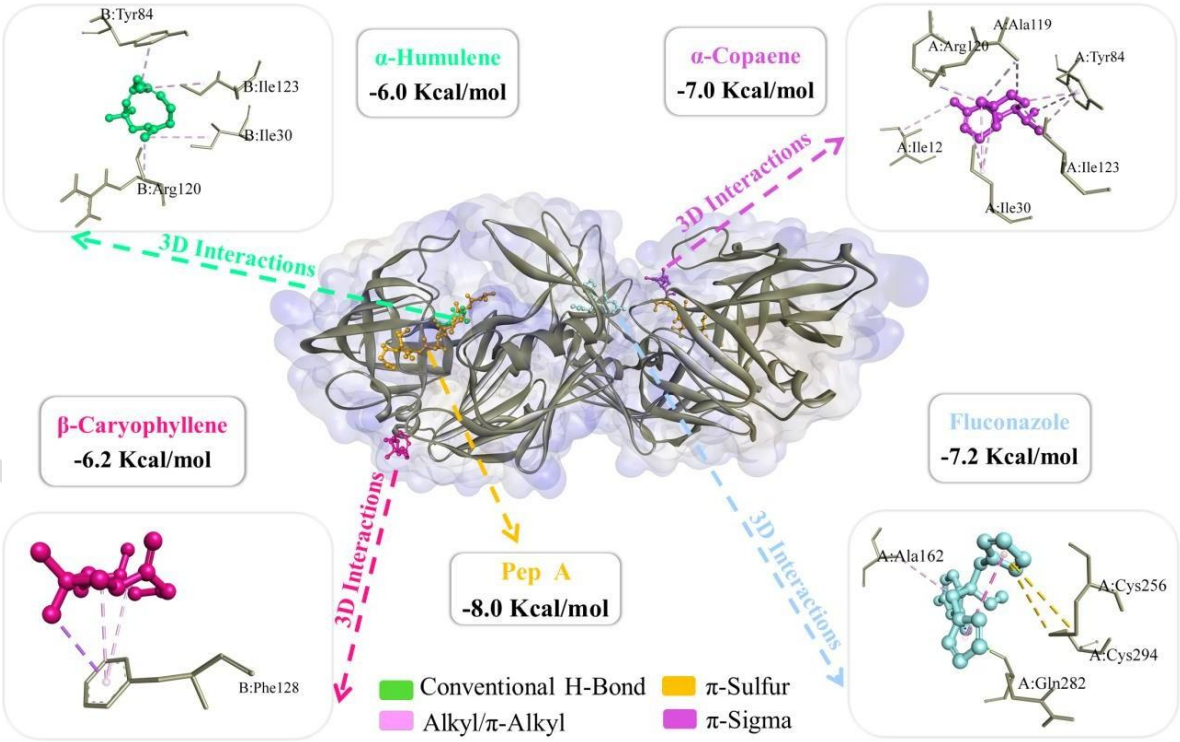

Fig.2.InteractioncomplexbetweenSAP5,α-Copaene,α-Humulene,β-caryophyllene,Fluconazole andtheco-crystallizedinhibitorPepstatinA(PepA).

4. CONCLUSION

*T.guianensis*leavesshowverypromisingantioxidant,anticholinesteraseandantifungal 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 havingseveral actions such as: antioxidant, anticholinesterase and antifungal. The *in silico*evaluationfor the inhibition of the acetylcholinesterase enzyme showed that all the complexes formedshowed hydrophobic interactions with the amino acid residue Trp 86, a residue present in theGNT binding site, indicating that all the ligands evaluated have a similar action to the druggalanthamine. The molecular docking results showed that the sesquiterpene compounds α-copaene,α-humulene and beta-caryophyllene have similaror superioraction to the drugphysostigmine,andalsosuggestapossiblesynergisticeffectofacetyleugenoleugenolcompoundstophysostigmine.

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, whichindicates its similar action to the aspartic protease inhibitor, in addition to the possibility of asynergisticeffectwiththedrugFluconazole.Wealsohighlightthatβ-caryophylleneinteracted with the assessed target, complexingata differentsite than the co-crystallizedPepA inhibitor (SAP5) and the Fluconazole control, which allows us to infer its possiblesynergistic effect with Fluconazole as a potential tool in the treatment of infections
fungalinfectionscausedby*Candidaalbicans*.However,thisproteintargetwasnotabletodemonstratetheactivityoftheknownanti fungaleugenol.

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

CONSENT (WHEREEVER APPLICABLE)

Not applicable to this submission.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Not applicable to this submission.

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