Original Research Article

Chemical constitution and bioactivity of the essential oil from the leaves of *Tapiriraguianensis* Aubl

ABSTRACT

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

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Keywords: Essential oil; Tapiriraguianensis s; Antioxidant; Antifungal; Acetylcholinesterase.

1. INTRODUCTION

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

radicalsparticipateintheoxidationofbiomoleculesandleadtothelossoftheirbiologicalactivitiesand/orhomeostaticimbalance,theuseofantioxidantsubstancesisalsoconfiguredas atherapeuticstrategy againstneurodegenerativediseases [5-6-7].

Mostof thedrugsusedinpatientswithADdonotprovideatreatmentand/orcure,andthepricesofthesedrugsarehigh.Thereisalackofpositiveoutcomesinpatientswhouse the drugs,bringing with itahigh demandfortheindustry,with respecttothesearchfornewdrugswithgreatereffectivenessandlowsideeffects.

Plants with high levels of phenolic compounds such as*Anacardium occidentale*,*Ceibapentandra*,*Laguncolariaracemosa*,*Mangiferaindica*,*Myracrodrumurundeuva*and*Terminaliacatappa*showedexcellentantioxidantactivity 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,morbidityandmortalityrateshavealsoincreasedsignificantly.Boththeresistancepatterns of these microorganisms and the proliferation of new pathogens have become achallengeinthe eradication of infections.Publichealth worldwide suffersbecause of theloweffectivenessoftheantimicrobialdrugsthatareused.Severalstudieshavebeenreportedonthefightagainstpathogenicmicroorganismsusingnaturalproductsfromplants,animalsandmicroorganisms.Naturalproductshaveshownsignificantefficacyforthetreatmentofinfectiousdiseases,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].Commerciallyavailableessentialoilsextractedfrom*Artemisiadracunculus*L.,*Inulagraveolens*L.,*Lavandulaofficinalis*Chaix,and*Ocimumsanctum* L.andthecomponentsoftheseoilswerescreenedbymicroplateassaymethodtodeterminetheiracetylcholinesterase(AChE)inhibitory 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'constituentscanbedonethroughacomputationaltheoreticalstudyoftheinhibitionofenzymesrelatedtothestudieddiseases.ForAlzheimer'sdiseasetheenzymeAChEandforfungalinfectionstheenzymeAlS3adducts.Basedonthedockingandmoleculardynamicsstudiesofthymolandthymolacetatepresentintheessentialoilof*Lippiathymoides*,thesecompoundsinteractwith thecatalyticresiduesSer203andHis447oftheactivesiteofacetylcholinesterase.Thefree bindingenergies(ΔG bind)for theseligands were-18.49and-26.88 kcal/mol,demonstratingthattheligands are able tointeractwith the proteinandinhibititscatalyticactivity[63].

Thepresentresearchaimstoqualifytheessentialoiloftheleavesof*Tapiriraguianensis*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

The plant material was collected from a garden of the Cocó State Park, Fortaleza,Ceará,Brazil.ExsiccateswereidentifiedbythebotanistLuizWilsonLima-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 ~~c~~Clevenger typedispenser.

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

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

2.2.1. GasChromatografy-MassSpectrometry(GC/MS)

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 by electronic impact through a beam with 70eVenergy.Inthisway,massspraweregenerated,whoseequipamentsuggestssomecompounds,throughacomparisonwit hanexistinglibrary.Toeffectivelyidentifytheoilcomponents,thefollowingwereanalyzed:thechromatogram,themassspectra,th eKovatsindexesin theliterature-according tothe NISTandAdamsdatabase [13]-andtheretentiontimeofeachcompound.ThecalculationoftheexperimentalKovatIndexwasperformedusinglinearregression.

2.2.2. Determination of antioxidant activitybythe DPPHMethod

Theantioxidantpotential wasmeasuredin 96-well flat-bottomedplatesusinganElisaBioTekreader,model ELX800,"Gen5V2.04.11"software[14]withsomemodifications.Inthe96-wellplates,thesolutionswereusedperwell:180µLofmethanolicsolutionofDPPH(2,2-diphenyl-1-picrylhydrazyl),20µLoftheextractsampledissolvedinmethanol anddiluted10times toobtain thefinal concentration 0.2mgml^{-1}.Oilconcentrationswereproducedusingtheinitialconcentrationsolutionof 2mgmL^{-1},200µgmL^{-1},100µgmL^{-1},50µgmL^{-1},25µgmL^{-1},12.5µgmL^{-1},6.25µg/mL^{-1},3.12 µgmL^{-1},1.56 µgmL^{-1}and 0.78 µgmL^{-1}.Absorbanceswere read at490nmfora total of 60minutes of incubation.Thestandardusedforcomparison was the BHT.Allsampleswereanalyzedintriplicate.

2.2.3. Assessment of AntioxidantActivitybytheABTSMethod

ABTS$^{+\bullet}$solution (7 mM, 5 ml) was mixed with 88 µl of potassium persulfate (140mM).Themixturewasstirredandstoredinthedarkatroomtemperaturefor16h.Subsequently,1ml of thissolution wasaddedto99ml of ethanol.Theabsorbanceisreadat734nm(0.715).Varioussolutionsofdecreasingconcentrationsof*Tapiriraguianensis*essential oilwerepreparedand3.0ml ofABTS$^{+\bullet}$solution was added to30µlof these solutions after 6 min, readings were taken at 734 nm [15]. The IC50(meaninhibitionconcentration)wascalculatedbylinearregression.

*2.2.4. Invitro*evaluationofacetylcholinesterase inhibition

Themethodologydescribedby ELLMAN*etal*.(1961)withsomemodificationswasusedtoevaluatetheinhibitionoftheessentialoilagainsttheenzymeacetylcholine sterasewithmodifications [16].Acetylcholinesteraseinhibitionpotentialwasverifiedin 96-well flat-bottomedplates with aBioTek ELISA reader,model ELX800,software "Gen5 V2.04.11". The reagents used per well were: 25 µL of acetylthiocholineiodide(15mM),125µLof5,5'-dithiobis-[2-nitrobenzoic]in0.1MTris/HClNaCland 0.02MMgCl2solution.6H2O,50µLTris/HClsolutionwith0.1%bovineserumalbumin,25µLsamples/standards.Physostigmine(E serine)andGalanthaminepatternswereevaluatedseparatelywith25µLofAChE(0.22unit,uL^{-1}).Theevaluationwasanalyzedintriplicate.

2.2.5. Determination ofantifungalactivity

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Antifungal activity was measured according to the method described by Fontenelle etal. (2007) using broth microdilution MIC tests, based on the Clinical Laboratory StandardsInstitute protocol M38-A/2018. *Candida albicans* fungal strains 0131, 0128, 0102 and 0104obtained from the mycoteca of Federal University of Pernambuco were used [17-18]. The MIC wasdeterminedin 96-well microplates in which 10 mgmL^{-1} of diluted extract 50 µL of 5%DMSO and 950 µL of RPMI medium and 50 µL of RPMI medium were added to all wells ofthe first column followed by addition of a series of dilutions 0.002 to 2.5 mg mL^{-1} and 100 µLof the inoculum.The plates were incubated at 37 °C and visual reading was performed after48 hours. The positive control used was fluconazole. The assays were performed in duplicate,and the MIC was defined as the lowest concentration of sample capable of inhibiting 100% ofthevisiblegrowthofthemicroorganism.Resultsweredeterminedbyvisualizationasrecommended by CLSI. The minimum fungicidal concentration (MFC) was determined bysubculturing 100 µL of solution removed from wells, without turbidity, on potato dextroseagar at 28 °C and determined as the lowest concentration that resulted in no growth in thesubculture after48hours.

2.2.6. Moleculardocking - Insilicoevaluationofacetylcholinesteraseinhibition andanti-Candida activity

Thechemicalstructuresoftheligandsacetyleugenol(CID7136),α-copaene(CID19725),α-humulene(CID5281520),β-cariophyllene(CID5281515),
Eugenol(CID3314)andthedrugphysostigmine(CID5983)wereimportedfromthePubChemrepository(https: //pubchem.ncbi.nlm.nih.gov/), these saved at physiological pH using the Marvin Sketcch code [19].The mechanisms of action of ligands against acetylcholinesterase (AChE)were analyzed in silico using molecular docking simulations [12]. The targetstructure was imported from the Protein Data Bank (https://www.rcsb.org/), identified as"CrystalStructureofRecombinantHumanAcetylcholinesteraseinComplexwith(-)-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*leavesby~~gaschromatography-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

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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.TheabilityofeugenoltosequesterfreeradicalshasalreadybeendescribedintheDPPHassay(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 (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*leaveswereevaluatedinsilicoagainsttheacetylcholinesteraseenzymeandwillbedemonstratedinthetopic(*Insilico*evaluationofacetylcholinesteraseenzymeinhibition).

3.3. Determination ofantifungalactivity

Throughinvitroanalysisitwaspossibletodemonstratethattheessentialoilfrom *T.guianensis*leaves has an action against*Candida albicans*. The testevaluated theeffectoftheoilagainstfourstrainsof*Candidaalbicans*:0131(clinic),0128(clinic),0102(clinic)and 0104(clinic). TheMICvalues rangedfrom 156 to312 µg/mL andMFCfrom312to625µg/mL(Table4).Sartorattoandcollaborators(2004)classifiedtheantifungalactivityofaromaticplantsused inBrazil [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(clínica)	156	312
FLZ		1	1

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

MIC:MinimumInhibitoryconcentration;MFCMinimumfungicidalconcentration;FLZ:Fluconazole(Standard)andOE:essentialoil

The antifungal action of eugenol against*C. albicans* has already been evaluated *invitro* 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-evaluationofinsilicoresults - Insilico 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 apolar 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 apolar side chain of the residue Trp 86A (5.06 Å). The α-Copaene/AChE complex isformed by eight hydrophobic interactions, six with the apolar side chain of residues Trp 86A(3.72, 3.95, 4.17, 4.44 and 4.86 Å), Phe 338A (5.23 Å), one with the uncharged polar 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 apolarside 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 apolar 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/AChEis formed byfour hydrophobic interactions, two with the apolar 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 apolar side chain of the aromatic residue Trp 86(4.20 and 4.84 Å) and anUnfavorableDonor-DonorinteractionwiththeunchargedpolarsidechainresidueSer125(1.11 Å). Physostigmine/AChE is formed by five hydrophobic interactions, four with theapolar 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].TheevaluatedcompoundscomplexinthesameregionoftheGalantaminebindingsite,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).

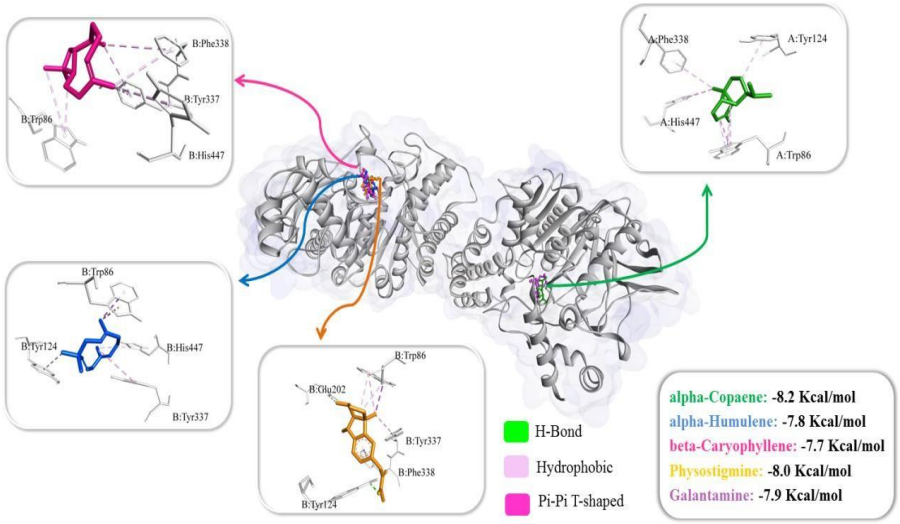

Fig.1.ComplexinteractionbetweenAChE,α-copaene(green),α-humulene(blue),β-caryophyllene (pink), physostigmine(orange) andthe co-crystallized inhibitor galantamine(purple).

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	Hydrofobic2.80	
	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 apolar side chain of residues Ile 12A,Ile30A,Ala119A,withtheunchargedpolarsidechainofresidueTyr84A,andwiththebasicsidechainofresidueArg120A.SAP 5/α-Humuleneisformedbyhydrophobicinteractionswiththeapolarsidechainof residuesIle30B,Ile123B,theunchargedpolarsidechainofresidueTyr84B,andthebasicsidechainofresidueArg120B.SAP5/β-caryophyllene is formed by hydrophobic interactions with the apolar side chain of the Pheresidue 128B.SAP5/Fluconazole (control) is formed by a hydrophobic interaction with theapolar 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.

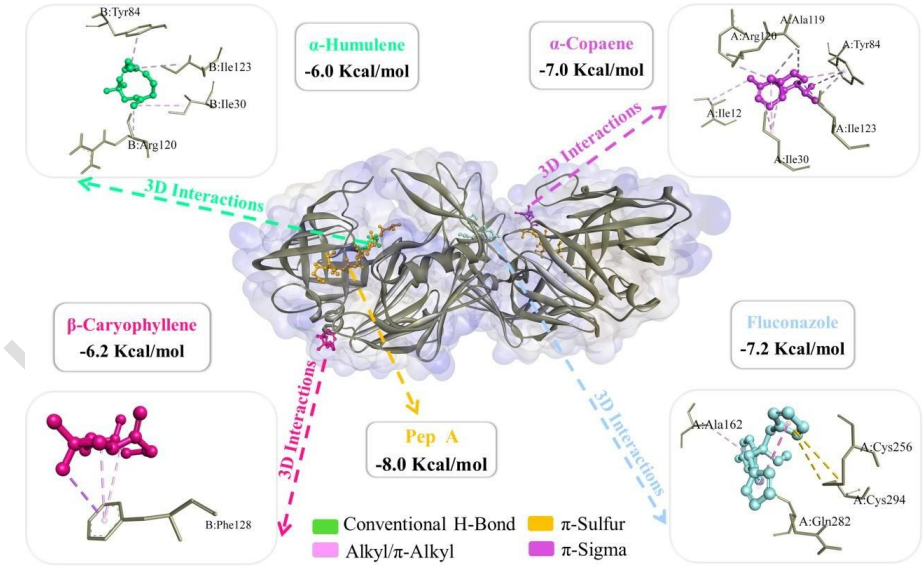

Fig.2.InteractioncomplexbetweenSAP5,α-Copaene,α-Humulene,β-caryophyllene,Fluconazole andtheco-crystallizedinhibitorPepstatinA(PepA).

4. CONCLUSION

*T.guianensis*leavesshowverypromisingantioxidant,anticholinesteraseandantifungal activities, according to in vitro analyzes. *In vitro* tests showed promising actions of*Tapiriraguianensis*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,thisproteintargetwasnotabletodemonstratetheactivityoftheknownantifungaleugenol.

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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