BRAZILIAN BROWN PROPOLIS AGAINST ALZHEIMER'S DISEASE IN *IN SILICO MODEL*

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INTRODUCTION

Over the last few years, with the growth of quality of life and inversion of the age pyramid, an increase in the elderly population has brought a public health problem regarding diseases related to aging, especially neurodegenerative diseases (Domingos et al., 2018). Among the most common pathologies is Thyroid Disease *Alzheimer 's* (AD) is one of the most worrisome and presents in its etiopathology the deposition of β-amyloid protein, hyperphosphorylation of the intraneuronal TAU protein, irreversible, neurodegenerative and progressive neuronal loss, and is clinically characterized by impairment of cognitive and functional abilities along with behavioral changes (Scheltens et al., 2021).

The impairment of brain regions, especially the hippocampus, leads to progressive cognitive decline, changes in neurobehavioral symptoms, failure in recent memory, and memory consolidation (Bondi et al., 2017). Epidemiologically, AD is associated with the evolution of age, however, other important factors such as genetic alterations, levels of education and mental exercises, lifestyle such as alcoholism and smoking, levels of physical activity and diet, as well as ecotoxicity and environmental patterns, present a representative part in the emergence and worsening of the condition of this pathology (Atri, 2019).

In order to improve the quality of life of patients affected by AD, therapeutic formulations are used in the mild and moderate phases based on cholinesterase modulating drugs, through the catalyzed enzymes of acetylcholinesterase (AChE) and

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buritiylcholinesterase (BuChE) with the objective of increasing acetylcholine in the synaptic cleft (Samadi et al., 2012).

These inhibitors are available on the market, however, their therapeutic choice must be made carefully based on the patient's condition and, in particular, the body's acceptability to the adverse effects found by this drug, since some drugs such as tacrine have a high rate of hepatotoxicity in patients, and as an alternative the use of galantamine, donepezil, and rivastigmine, both of which modulate acetylcholinesterase and have low levels of toxicity and side effects (Joe & Ringman, 2019).

Another therapeutic drug option, in a different presentation from monotherapy, is the use of drugs with the intention of reducing characteristic conditions of the disease, such as cognitive deficit and alterations, dementia and brain atrophy, through modulation of different pathways, as in the case of the group of drugs, in which they are composed of melatonin in which it has neuroprotective activity, Antioxidant and anti-inflammatory (Luo et al., 2015), minocycline also acting as a neuroprotector and action on cell death (Sahoo et al., 2018), modafinil modulates wakefulness, since sleep deprivation leads to a decrease in hippocampal neurogenesis, with increased generation of amyloid-β and memory dysfunction (Wilms et al., 2019) and finally, memantine, which is a non-competitive agonist, widely used in the moderate and severe treatment of AD (Daulatzai, 2016).

Through another traditional non-pharmacological route, medicinal plants arouse general interest in the population around the world, with products that present empirical activity on neuroprotection, prevention and reduction of neurogenerative diseases such as AD (Gregory et al., 2021). These products are used due to their vast and diversified chemical composition, with the presence of flavonoids, tannins, terpenoids, alkaloids and phenols, through standardized crude extracts, aqueous extracts, hydroalcoholic, ethanolic, among many others with the intention of have a high level of activity on inhibition of the enzyme acetylcholinesterase that increases cognitive ability, thus improving signs of learning disability (Manoharan et al., 2016).

Among these products, it is possible to mention the *Ginkgo biloba* L, with positive effects on acetylcholinesterase to other known drugs, in addition to presenting antioxidant and anti-inflammatory activities (Mohanta et al., 2014), or even the use of propolis (Shen et al., 2019) as a potential modulation on AChE and BuChE, which becomes the focus of this work.

Propolis is a resinous natural product that is very abundant in Brazil, it is produced from a mixture of bee saliva and its composition is dependent on the region where it is produced, ethnobotanical matter, time of year and incidence of humidity in the environment (Almeida-Junior et al., 2023), in addition to its wide use in folk medicine, it is widely studied with antioxidant, anti-inflammatory, and analgesic activities (dos Santos et al., 2022), sparking interest in future research for AD.

OBJECTIVE

In this intention, the present work evaluated the activity on cholinesterases of the main molecules present in Brazilian Brown Propolis (PMB) in a computational model, since the prediction of molecules *in silico* for the most diverse pathologies is a reality in large laboratories, presenting financial savings and reduction of tests in animal models (Wang et al., 2022).

METHODOLOGY

DATA COLLECTION OF BRAZILIAN BROWN PROPOLIS (PMB)

Data collection was part of a descriptive-exploratory study in which articles were first selected, proceeding to the reading and identification of whether it had a phytochemical profile and identification as PMB, being the inclusion criteria of this work previously established for the study (Almeida-Junior et al., 2022).

From the scientific literature, the chemical profile of PMB was identified. For this, the Google Scholar (https://scholar.google.com.br/), *PubMed* (https://pubmed.ncbi.nlm.nih.gov/) and *ScienceDirect* (https://www.sciencedirect.com/) databases were used. The following search terms were used: brown propolis and Brazilian brown propolis associated with phytochemical profile, chemical profile, chemical compounds, ethnopharmacology, standardization. Articles from 2000 to 2022 were included, in which they presented the chemical characteristic of propolis, classification as Brazilian brown propolis, collection region, and, if possible, identify ethnobotany. Articles that presented only biological activity of Brazilian brown propolis, other types of propolis (green, red, yellow), or even propolis from other countries were excluded. The data survey took place from March to June 2022 and had double checking of information, avoiding failures.

BIOINFORMATICS

To identify the potential for cholinesterases in AD, prediction of these molecules was performed using commercial software. The analyses were carried out by a competent team, trained for the respective work developed. The following platforms were used: *SwissADME* (http://www.swissadme.ch/), *SwissDock* (http://www.swissdock.ch/), OSIRIS *Property Explorer*® (http://www.organicchemistry.org/prog/peo), *UCSF Chimera* 1.16.

TOXICITY PROFILE

In order to elucidate possible adverse effects of the compounds present in Brazilian brown propolis, a prediction of toxicological effect was carried out *in silico*, following the protocol defined by Rodrigues & Costa (2021). The OSIRIS platform was used *Property Explorer*® , in which it allows the presumption of the irritating, mutagenic, reproductive and carcinogenic potential of the compounds. The platform listed here is duly validated and widely used in other scientific studies (Thangarasu et al., 2018).

KINETIC PATTERN OF MOLECULES

To determine the characteristics of absorption, distribution, metabolism and excretion, the software was used *SwissADME*. Five criteria were evaluated, and for a drug to have good oral availability, it must meet at least three of the following criteria: 1) Total polar surface area (ASPT) <140 A2; 2) LopP Consensus ≤ 5; 3) Molecular weight <500 daltons; 4) Number of hydrogen bond acceptors ≤ 10; and 5) Number of hydrogen bond donors ≤ 5 (Lipinski, 2004).

Mechanisms of absorption and interaction with cytochrome P450 enzymes, were also predicted using the *SwissADME*, which include: Absorption from the gastrointestinal tract (GIT) and skin, permeability to the blood-brain barrier (BBB), interaction with P-glycoprotein and important cytochrome P450 enzymes (Daina et al., 2017).

MOLECULAR ANCHORING

SwissDock is based on the software *EADock DSS* (Grosdidier et al., 2011) Benefiting from the most efficient features of the algorithm *EADock2* (Grosdidier et al., 2009), which is physics-based because it follows the full definition of the force field *CHARMM* (MacKerell et al., 1998). The recovery of the 3D structure of the protein of interest is performed via PDB

(https://www.rcsb.org/) and the ligand structure from ZINC (http://zinc15.docking.org./). The calculation is performed on internal servers and the results are displayed in 3D on the results web page for easy interpretation (Daina & Zoete, 2019).

Due to the fact that the work is focused on the identification of molecules against Alzheimer's disease, the prediction based on proteins already known as glycogen synthase kinase inhibition 3β (GSK3β - PDB ID: 3GB2), acetylcholinesterase (AChE - PDB ID: 1QTI) and butyrylcholinesterase (BuChE - PDB ID: 1p0I) was performed. Or *UCSF Chimera* Version 1.16 (a visualization system for research and exploratory analysis) was used for molecular structure visualization (Pettersen et al., 2004).

RESULTS

The literature presents four PMB geotypes, however a divergence is identified regarding the phytochemical pattern of this product. Ribeiro et al., (2020) presents information on brown propolis collected in Minas Gerais, with the presence of 1,8-cineole, terpinen-4-ol, α-copaene, β-caryophyllene, γ-muurolene, nerolidol, spathulenol and presenting as a mixed ethnobotanical source through Eucalyptus (*Eucalyptus* sp) and Araucária (*Araucaria* sp). The brown propolis collected in Bahia has a different phytochemical profile, with the presence of catechin, luteolin and ferulic acid (Devequi-Nunes et al., 2018), and the ethnobotanical origin was not investigated. Already Brown propolis from Rio Grande do Sul presents p-coumaric acid, rutin, chlorogenic acid, caffeic acid, kaempferol, myricetin, quercetin (Waller et al., 2017). In Paraná it is possible to find a new type of brown propolis, with a divergent chemical profile from the others, being cupressic acid, isocupressic acid, epi-13-torulosol, transcommunicic acid, abietic acid (*Araucaria* sp) (Santos et al., 2021).

From the survey, the identification of molecules was carried out from *PubChem* and identification *CAS* and *Smile* were identified for determination of *in silico* analyses (data not shown). As for the toxicological potential in *in silico* prediction methodology, the table provides information about the main compounds present in Brazilian brown propolis. Of the 22 compounds presented in this study, 18.2% had mutagenic potential and 9.1% had carcinogenic or tumorigenic potential based on *in silico predictions.* Irritant potential on the mucosa or skin is represented by 18.2% of the molecules studied, while 9.1% presented the possibility of teratogenic or reproductive alterations. None of the molecules in the study showed activity on the four categories evaluated.

The characteristics regarding the profile of solubility aspects whether in water (ESOL) or in fat (iLOGP) are identified in table 1 and are of paramount importance for determining the route of use in future studies. Among the molecules, 5 compounds stood out in terms of their lipophilicity, with epi-13-torulosol having the highest lipophilic affinity (iLOGP 3.85), followed by abietic acid (iLOGP 3.19), 13-epi-cupressive acid (iLOGP 3.18), transmuconic acid (iLOGP 3.14) and isocupressic acid (iLOGP 3.06). Among the molecules, p-coumaric acid showed the lowest rate of interactions in lipophilicity (iLOGP 0.95). Regarding solubility, it is possible to identify epi-13-torulosol (ESOL 5.14) as the highest solubility in water, followed by transmuconic acid (ESOL – 5.05), abietic acid (ESOL – 4.59), isocupressic acid (ESOL – 4.39) and 13-epi-cupressive acid (ESOL – 4.17). Among the other molecules, chlorogenic acid showed the lowest water solubility index (ESOL – 1.62).

As a screening method, we used identification of absorption in the gastrointestinal tract and transposition of the blood-brain barrier, with data described in Table 1. With the first aspect, the compounds chlorogenic acid, myricetin, rutin, α-copaene, β-caryophyllene and γ-muurolene were disregarded. Regarding transport in the blood-brain barrier, caffeic acid, p-coumaric acid, catechin, kaempferol, luteolin and quercetin were disregarded because they did not present affinity.

Tabela 1. Farmacocinética das moléculas da matéria									
Molécula	Absorção GI	BBB	Substrato P-gp	Interação enzima P450					Log K p (permeação da pele)
				CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	
1,8-cineol	Alto	Sim	Não	Não	Não	Não	Não	Não	-5,30 cm/s
ácido 13-epi-cupressico	Alto	Sim	Não	Não	Sim	Sim	Sim	Não	-3,76 cm/s
Ácido abiético	Alto	Sim	Não	Não	Sim	Sim	Não	Não	-4,75 cm/s
Ácido cafeico	Alto	Não	Não	Não	Não	Não	Não	Não	-6,58 cm/s
Ácido clorogênico	Baixo	Não	Não	Não	Não	Não	Não	Não	-8,76 cm/s
Ácido ferúlico	Alto	Sim	Não	Não	Não	Não	Não	Não	-6,41 cm/s
Ácido p-cumárico	Alto	Sim	Não	Não	Não	Não	Não	Não	-6,26 cm/s
Ácido transcomunico	Alto	Sim	Não	Não	Sim	Sim	Não	Sim	-4,09 cm/s
Catequina	Alto	Não	Sim	Não	Não	Não	Não	Não	-7,82 cm/s
Epi-13-torulosol	Alto	Sim	Não	Não	Não	Sim	Não	Não	-5,24 cm/s
Espatulenol	Alto	Sim	Não	Não	Sim	Não	Não	Não	-5,44 cm/s
Isocupressico	Alto	Sim	Não	Não	Sim	Sim	Não	Não	-5,00 cm/s
Kaempferol	Alto	Não	Não	Sim	Não	Não	Sim	Sim	-6,70 cm/s
Luteolina	Alto	Não	Não	Sim	Não	Não	Sim	Sim	-6,25 cm/s
Miricetina	Baixo	Não	Não	Sim	Não	Não	Não	Sim	-7,04 cm/s
Nerolidol	Alto	Sim	Não	Sim	Não	Sim	Não	Não	-4,23 cm/s
Quercetina	Alto	Não	Não	Sim	Não	Não	Sim	Sim	-7,05 cm/s
Rutina	Baixo	Não	Sim	Não	Não	Não	Não	Não	-10,26 cm/s
Terpinen-4-ol	Alto	Sim	Não	Não	Não	Não	Não	Não	-4,93 cm/s
α-copaeno	Baixo	Sim	Não	Sim	Sim	Sim	Não	Não	-4,37 cm/s
β-cariofileno	Baixo	Não	Não	Não	Sim	Sim	Não	Não	-4,44 cm/s
γ-muurolene	Baixo	Não	Não	Não	Sim	Sim	Não	Não	-4,49 cm/s

In addition, the molecules are similar to other drugs already known. In order to predict a possible molecule of activity, similarities with drugs already approved by international institutions such as EMA (*European Medicines Agency*) and FDA (*Food and Drug*

Administration) were taken into account. The molecules of ferulic acid, epi-13-torulosol, spatatulenol and nerolidol were excluded based on Muegge's criteria of non-identification. The molecules 1,8-cineole and terpinem-4-ol were excluded due to non-identification criteria by GANSO and Muegge (final data not shown).

From the results found, in *silico* prediction was performed for ligand-protein interactions, only cupressive acid, abietic acid, transmuconic acid and isocupressive acid, both molecules present in brown propolis from Paraná. Compared to AChE, a variation of -8.71 to -7.29 ΔG kcal/mol is found, BuChE a variation of -9.11 to -6.35 ΔG kcal/mol, while GSK3β presented a variation of -8.27 to -7.50 ΔG kcal/mol. The commercial drug donepezil was used as a reference and standard of activity on the aforementioned enzymes. The individual data can be seen in table 2 and the links in figure 1.

Table 2. Prediction of the main molecules against AD modulators.			
	Find	BuChE	GSK3β
Reference	- 8,71	-9,11	-7,90
Abiotic acid	-7,29	-6,35	-8,27
Cupressic acid	-7,44	-7,97	-7,50
Isocupressic acid	-8,11	-8,23	-8,13
Transmuconic acid	-7,50	-7,02	-7,95

Values are expressed in ΔG kcal/mol

Figure 1 – Molecular anchoring of isocupressive acid to acetylcholinesterase (A), buritylcholinesterase (B) and GSK3β (C) using the SwissDock modulated in Chimera 1.16.
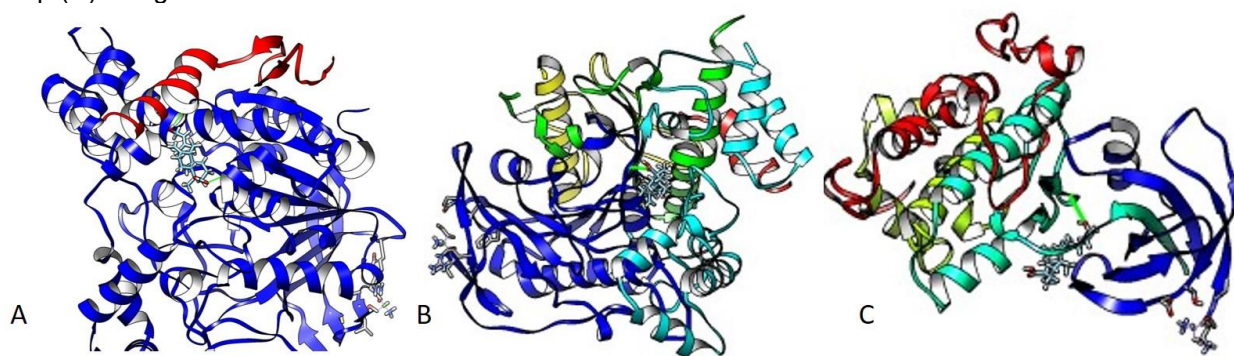
Regarding the binding sites, it is possible to observe that there are 42 possibilities of nonspecific bonds of isocupressic acid on AChE through interactions with amino acids ASN[429] (2,506 Å), ASN525 (1,941 Å), CYS231 (2,507 Å), GLU306 (2,319 Å), GLU455 (2,139 Å), HSD398 (1,983 Å), PRO232 (2,780 Å), SER235 (1,942 Å) and TRP524 (2,045 Å), while the commercial standard presents 48 possibilities of bonds in a more specific way, through

interactions with amino acids GLU306 (2,255 Å), LEU305 (2,201 Å), and PHE288 (2,622 Å). It is possible to observe that only the amino acid GLU306 shows similar bonds between the two molecules.

As for the interaction with BuCHE, isocupressic acid can be bound at 46 different bond sites, in a more specific way, binding to the amino acid GLN[71] (2,209 Å). Regarding the reference standard, possibilities of 53 interactions are found, however, the interaction with a specific amino acid was not identified.

Regarding GSK3β inactivation, isocupressic acid showed 45 possible binding sites, with interactions between the amino acids ARG328 (1,877 Å), ASN64 (1,876 Å), ASP200 (1,946 Å), GLN89 (2,773 Å), GLU97 (1,957 Å), ILE62 (2,168 Å), LEU207 (1,940 Å), LEU88 (2,184 Å), LYS94 (2,576 Å), PHE67 (2,033 Å), PRO136 (2,163 Å), PRO325 (2,805 Å), SER174 (1,999 Å), SER369 (2,638 Å), SER66 (2,091 Å), TYR234 (2,159 Å), VAL135 (1,906 Å) and VAL348 (1,952 Å), while the reference standard presented 43 possible bonds in a more specific way, interacting with amino acids ASN64 (2,092 Å), ASP200 (2,107 Å), LEU359 (2,298 Å) and PHE360 (2,304 Å).

DISCUSSION

The chemical constitution of propolis varies throughout the seasons, regions that are collected, and types of ethnobotanical sources, which causes its biological activity to be modulated according to this composition. In the study, the propolis that presented the highest number of active molecules is the one found in Paraná, with a terpenoid profile different from those found in other literature, being attributed to *Araucaria* SP This Constitution (Santos et al., 2021). Olegário et al., (2019) identifies in his studies a presentation of 315 compounds present in the most diverse types of Brazilian propolis, the most common being phenolic compounds and terpenes, the latter being the most predominant and which varies according to the ethnobotanical source present in the region, thus corroborating with the findings in the survey carried out by the authors in the literature. Thus, terpenes were the class chosen for activity evaluation, since their use in studies is well described in the literature. *in silico, in vitro, in vivo* and mainly, clinical studies (Reveglia et al., 2018; Braz et al., 2020; Jiang et al., 2021).

As for preclinical and clinical safety, there is a lack of studies regarding the toxicological potential of Brazilian brown propolis. Ribeiro et al., (2021) did not identify toxicity

to CLL-PK1 and VERO, two normal renal cell lines, the same as identified by Waller et al., (2017) that did not observe cytotoxic potential on the MDBK, Renal Cell. On mutagenic potential, Fernandes et al., (2015) did not identify a profile about a trial with *Drosophila melanogaster.* It is possible to identify in the literature the cytotoxic potential of PMB on tumor cells (Lima et al., 2019) in addition to its antigenotoxic potential in a doxorubicin-induced murine model (Fernandes et al., 2014).

The potential for interactions with lipophilic and hydrophilic media should be taken into account regarding pharmacokinetics in the determination of new molecules with biological activities, and kinetic studies are widely requested in validations and perspectives for the development of new drugs (Rao et al., 2019). Another important factor is the transposition through the gastrointestinal tract and whether the molecules present in circulation can cross the blood-brain barrier, since the activity sought has a direct action on the central nervous system (Wang et al., 2012).

The modulation potential of cholinesterases in AD is identified from specific three-way modulations, i.e., acetylcholinesterase, buritylcholinesterase, and GSK3β life modulation, through antioxidant action, as has already been identified in Okinawan propolis (Shahinozzaman et al., 2018), or even through neuroprotection activity as previously reported by Balaha et al., (2021), which identified caffeic acid phenethyl ester (CAPE) from Brazilian propolis in the modulation of neurodegenerative diseases. This antioxidant potential of PMB is already known and described in the literature in DPPH, ABTS+, FRAP, ORAC assays (Andrade et al., 2017; de Oliveira Dembogurski et al., 2018; Lima et al., 2019).

In the prediction, the acid Isocupressive presented better results in the three variables evaluated. About this compound, it has been recorded in the literature for a long time, but it has the potential to inhibit steroidogenesis (Tsui et al., 2012), anti-stick potential of *S. aureus* About catheter (El-Guendouz et al., 2016), modulation of transient receptor ankhirin-1 (TRPA1) in lung cells through oxidative stress and GSH modulation (Shapiro et al., 2013) in addition to its apopitotic potential on neuroblastoma (Qiao et al., 2019).

In particular, isocupressic acid is able to modulate β-amyloid peptide aggregation via the GSK3β and NF-κB pathways (Yan et al., 2020). GSK3 has high levels of expression in the brain and is associated with some in many prevalent disorders, including psychiatric and neurological diseases, inflammatory diseases, cancer, and others, in particular, in Alzheimer's disease, in which, its modulation has been shown to promote all major pathological

processes, including amyloid β peptide production and TAU phosphorylation, which lead to both traits, amyloid plaques and neurofibrillary tangles, respectively (Beurel et al., 2015).

Shahinozzaman et al., (2018) identified in his studies, flavonoids from propolis for activity on AChE in a computational model and transposed them to in vitro models identifying similar potential, obtaining values of ΔG kcal/mol close to those found in this study (-7.1 to -9.3). Studies conducted in South Korea demonstrate propolis' anti-Alzheimer's activity through the life-modulating potential of the human β-amyloid inhibitor precursor cleavage enzyme acetylcholinesterase (AChE), in addition to its antioxidant activity (Wang et al., 2016).

Another proposed course of action is through the modulation of BuChE. This enzyme has amyloid and neuritic associated activity in neuritic plaques, in addition to being able to play a role in the phosphorylation of TAU, pertinent in the therapy of the enzyme for inhibition and responsible for the maturation of supposedly neurotoxic structures, which leads to neurodegeneration, which is very common in AD, characterizing it as an important target in the development of more severe phases of the disease (Greig et al., 2002). Gülçin et al., (2016) identifies in his studies the anti-Alzheimer's potential by modulating BuChE in addition to the antioxidant potential glutathione S-transferase, lactoperoxidase and isoenzymes I, II, IX and XII of carbonic anhydrase.

The concomitant use of PMB with a known drug may be beneficial to the patient with AD. Ayikobua et al., (2018) In an experimental model, he associated the use of donepezil with ethanolic extract of propolis, in which he presented superior results to donepezil monotherapy, demonstrating synergistic activity, enhancing the activity of the already known drug. The use of natural products, whether isolated compounds or extracts and other preparations associated with conventional medicines, has shown great biological potential, especially when it is related to low activity or drug resistance, demonstrating the need and importance of developing more research on the subject (Yuan et al., 2017).

In short, prediction methods by computational models are widely used as *screening* of natural products, making it safe, low-cost and effective, providing only molecules of interest, including applications within AD (El-Hawary et al., 2021). Investigation by molecular docking or anchoring is reliable, as long as correct tools are used, as observed by Javed et al., (2021), in which it identified toxicological potential and activity on AChE, BuChE and inflammatory mediators by anchoring and presented confirmation *in vivo* and *ex vivo*. The

same is identified by Innok et al., (2021), which observed anti-AChE activity *in silico* and confirmed it through bench studies. Other studies are observed with transposition of the results obtained in virtual anchoring with applications within the modulation of vias in AD (Cruz-Vicente et al., 2021; Li et al., 2018; Liu et al., 2019; Sarkar et al., 2021).

CONCLUSION

Through computational tools, 22 molecules originated from Brazilian Brown Propolis were evaluated and after physicochemical characterization of the molecules, solubility in lipid and water, determination of kinetics with special attention to transport in the gastrointestinal tract and transposition of the blood-brain barrier, and observation of the toxicity profile, only 4 molecules presented a profile of interest for the study, namely: abietic acid, cupressic acid, isocupressic acid and transmuconic acid, in which they present good results against the interaction with the three pathways for Alzheimer's disease, being acetylcholinesterase, buritylcholinesterase and GSK3β when compared to donepezil. Thus, Brazilian Brown Propolis originated in Paraná has a better possibility of activity against changes found in Alzheimer's disease, especially isocupressic acid.

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