Revista ARACÊ
ISSN: 2358-2472

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

https://doi.org/10.56238/arev6n4-148

Submitted on: 11/10/2024 **Publication date:** 12/10/2024

Laura Bressan Callafatti[1], Milena Souza Tavares[2], Jhonatan Felipe dos Santos[3], Ricardo Andrade Furtado[4] and Silvio de Almeida Junior[5].

ABSTRACT

INTRODUCTION: Over the last few years, with the growth in 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, Alzheimer's Disease (AD) is one of the most worrisome and has 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 the impairment of cognitive and functional abilities along with behavioral changes (Scheltens et al., 2021).

Keywords: Brown Propolis. Alzheimer's disease. Model in Silico. Antioxidant Properties.

[1] Bachelor in Biomedicine, University of Franca, Brazil
E-mail: laura.bressan@hotmail.com
[2] Bachelor in Biomedicine, University of Franca, Brazil
University of Franca, Brazil
Email: tavares_mih@hotmail.com.br
[3] Laboratory of Biosciences and Health, Graduate Program in Animal Science, University of Franca, Brazil
Email: jhonatanfelipe957@gmail.com
[4] Laboratory of Animal Science, Graduate Program in Animal Science, and Graduate Program in Health Promotion, University of Franca, Brazil.
E-mail: Ricardo.furtado@unifran.edu.br
[5] Laboratory of Biosciences and Health, State University of Minas Gerais, Brazil. Animal Science Laboratory, Graduate Program in Animal Science, University of Franca, Brazil
E-mail: silvioalmeidajr@yahoo.com.br

Revista ARACÊ
ISSN: 2358-2472

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 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),

Revista ARACÊ
ISSN: 2358-2472

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).

Revista ARACÊ
ISSN: 2358-2472

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 you to presume the irritating, mutagenic, reproductive and carcinogenic potential of compounds. The platform listed here is duly validated and widely used in other scientific studies* (Thangarasu et al., 2018).

Revista ARACÊ
ISSN: 2358-2472

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

Revista ARACÊ
ISSN: 2358-2472

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 has 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-

Revista ARACÊ
ISSN: 2358-2472

coumaric acid, catechin, kaempferol, luteolin and quercetin were disregarded because they did not present affinity.

Table 1. Pharmacokinetics of matter molecules

Molecule	GI absorption	BBB	P-gp substrate	P450 Enzyme Interaction					Log K p (skin permeation)
				CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	
1,8-cineole	High	Yes	No	No	No	No	No	No	-5.30 cm/s
13-epi-cupressic acid	High	Yes	No	No	Yes	Yes	Yes	No	-3.76 cm/s
Abiotic acid	High	Yes	No	No	Yes	Yes	No	No	-4.75 cm/s
Caffeic acid	High	No	No	No	No	No	No	No	-6.58 cm/s
Chlorogenic acid	Low	No	No	No	No	No	No	No	-8.76 cm/s
Ferulic acid	High	Yes	No	No	No	No	No	No	-6.41 cm/s
P-coumaric acid	High	Yes	No	No	No	No	No	No	-6.26 cm/s
Transcommunicating Acid	High	Yes	No	No	Yes	Yes	No	Yes	-4.09 cm/s
Catechin	High	No	Yes	No	No	No	No	No	-7.82 cm/s
Epi-13-torulosol	High	Yes	No	No	No	Yes	No	No	-5.24 cm/s
Spatulenol	High	Yes	No	No	Yes	No	No	No	-5.44 cm/s
Isocupressic	High	Yes	No	No	Yes	Yes	No	No	-5.00 cm/s
Kaempferol	High	No	No	Yes	No	No	Yes	Yes	-6.70 cm/s
Luteolin	High	No	No	Yes	No	No	Yes	Yes	-6.25 cm/s
Myricetin	Low	No	No	Yes	No	No	No	Yes	-7.04 cm/s
Nerolidol	High	Yes	No	Yes	No	Yes	No	No	-4.23 cm/s
Quercetin	High	No	No	Yes	No	No	Yes	Yes	-7.05 cm/s
Rutin	Low	No	Yes	No	No	No	No	No	-10.26 cm/s
Terpinen-4-ol	High	Yes	No	No	No	No	No	No	-4.93 cm/s
α Copaene	Low	Yes	No	Yes	Yes	Yes	No	No	-4.37 cm/s
β-caryophyllene	Low	No	No	No	Yes	Yes	No	No	-4.44 cm/s
γ-muurolene	Low	No	No	No	Yes	Yes	No	No	-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.

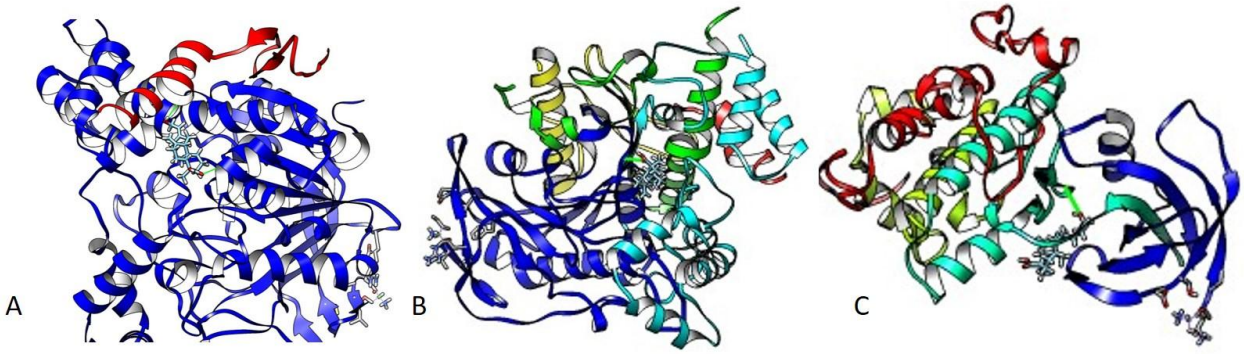
Revista ARACÊ
ISSN: 2358-2472

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 modulated SwissDock 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 ASN429 (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 binding sites, in a more specific way, binding to the amino acid GLN71 (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

Revista ARACÊ
ISSN: 2358-2472

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).

Revista ARACÊ
ISSN: 2358-2472

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

Revista ARACÊ
ISSN: 2358-2472

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

Revista ARACÊ
ISSN: 2358-2472

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.

Revista ARACÊ
ISSN: 2358-2472

REFERENCES

1. Almeida-Junior, S. de, Nicobue, V. R., & Santos, J. C. dos. (2022). Uso da ozonioterapia e medicina Ayurveda como práticas complementares no tratamento de infecção por Sars-CoV-2. *REVISE - Revista Integrativa em Inovações Tecnológicas nas Ciências da Saúde*, *9*(fluxocontinuo), Artigo fluxocontinuo. https://doi.org/10.46635/revise.v9ifluxocontinuo.2625

2. Andrade, J. K. S., Denadai, M., de Oliveira, C. S., Nunes, M. L., & Narain, N. (2017). Evaluation of bioactive compounds potential and antioxidant activity of brown, green and red propolis from Brazilian northeast region. *Food Research International*, *101*, 129–138. https://doi.org/10.1016/j.foodres.2017.08.066

3. Atri, A. (2019). The Alzheimer's Disease Clinical Spectrum: Diagnosis and Management. *The Medical Clinics of North America*, *103*(2), 263–293. https://doi.org/10.1016/j.mcna.2018.10.009

4. Ayikobua, E. T., Semuyaba, I., Eze, D. E., Kalange, M., Nansunga, M., Okpanachi, A. O., & Safiriyu, A. A. (2018). Combined Donepezil and Ethanolic Extract of Propolis Improved Memory Better Than Donepezil and Propolis Monotherapy in Wild Type Drosophila melanogaster. *Evidence-based Complementary and Alternative Medicine : eCAM*, *2018*, 3717328. https://doi.org/10.1155/2018/3717328

5. Balaha, M., De Filippis, B., Cataldi, A., & di Giacomo, V. (2021). CAPE and Neuroprotection: A Review. *Biomolecules*, *11*(2), 176. https://doi.org/10.3390/biom11020176

6. Beurel, E., Grieco, S. F., & Jope, R. S. (2015). Glycogen synthase kinase-3 (GSK3): Regulation, actions, and diseases. *Pharmacology & therapeutics*, *0*, 114–131. https://doi.org/10.1016/j.pharmthera.2014.11.016

7. Bondi, M. W., Edmonds, E. C., & Salmon, D. P. (2017). Alzheimer's Disease: Past, Present, and Future. *Journal of the International Neuropsychological Society: JINS*, *23*(9–10), 818–831. https://doi.org/10.1017/S135561771700100X

8. Braz, J. de V. C., Carvalho Nascimento Júnior, J. A., & Serafini, M. R. (2020). Terpenes with Antitumor Activity: A Patent Review. *Recent Patents on Anti-Cancer Drug Discovery*, *15*(4), 321–328. https://doi.org/10.2174/1574892815666201002162315

9. Cruz-Vicente, P., Passarinha, L. A., Silvestre, S., & Gallardo, E. (2021). Recent Developments in New Therapeutic Agents against Alzheimer and Parkinson Diseases: In-Silico Approaches. *Molecules (Basel, Switzerland)*, *26*(8), 2193. https://doi.org/10.3390/molecules26082193

10. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), Artigo 1. https://doi.org/10.1038/srep42717

11. Daina, A., & Zoete, V. (2019). Application of the SwissDrugDesign Online Resources in Virtual Screening. *International Journal of Molecular Sciences*, *20*(18), 4612. https://doi.org/10.3390/ijms20184612

Revista ARACÊ
ISSN: 2358-2472

12. Daulatzai, M. A. (2016). Pharmacotherpy and Alzheimer's Disease: The M-Drugs (Melatonin, Minocycline, Modafinil, and Memantine) Approach. *Current Pharmaceutical Design*, *22*(16), 2411–2430. https://doi.org/10.2174/1381612822666160203142111

13. de Almeida-Junior, S., Ferraz, M. V. F., de Oliveira, A. R., Maniglia, F. P., Bastos, J. K., & Furtado, R. A. (2023). Advances in the phytochemical screening and biological potential of propolis. *Fundamental & Clinical Pharmacology*, *37*(5), 886–899. https://doi.org/10.1111/fcp.12898

14. de Oliveira Dembogurski, D. S., Silva Trentin, D., Boaretto, A. G., Rigo, G. V., da Silva, R. C., Tasca, T., Macedo, A. J., Carollo, C. A., & Silva, D. B. (2018). Brown propolis-metabolomic innovative approach to determine compounds capable of killing Staphylococcus aureus biofilm and Trichomonas vaginalis. *Food Research International*, *111*, 661–673. https://doi.org/10.1016/j.foodres.2018.05.033

15. Devequi-Nunes, D., Machado, B. A. S., Barreto, G. de A., Rebouças Silva, J., da Silva, D. F., da Rocha, J. L. C., Brandão, H. N., Borges, V. M., & Umsza-Guez, M. A. (2018). Chemical characterization and biological activity of six different extracts of propolis through conventional methods and supercritical extraction. *PLoS ONE*, *13*(12). https://doi.org/10.1371/journal.pone.0207676

16. Domingos, J., Keus, S. H. J., Dean, J., de Vries, N. M., Ferreira, J. J., & Bloem, B. R. (2018). The European Physiotherapy Guideline for Parkinson's Disease: Implications for Neurologists. *Journal of Parkinson's Disease*, *8*(4), 499–502. https://doi.org/10.3233/JPD-181383

17. dos Santos, F. F., Morais-Urano, R. P., Cunha, W. R., de Almeida, S. G., Cavallari, P. S. dos S. R., Manuquian, H. A., Pereira, H. de A., Furtado, R., Santos, M. F. C., & Amdrade e Silva, M. L. (2022). A review on the anti-inflammatory activities of Brazilian green, brown and red propolis. *Journal of Food Biochemistry*, *6*(n/a), e14350. https://doi.org/10.1111/jfbc.14350

18. El-Guendouz, S., Aazza, S., Lyoussi, B., Bankova, V., Lourenço, J. P., Costa, A. M. R., Mariano, J. F., Miguel, M. G., & Faleiro, M. L. (2016). Impact of Biohybrid Magnetite Nanoparticles and Moroccan Propolis on Adherence of Methicillin Resistant Strains of Staphylococcus aureus. *Molecules*, *21*(9), 1208. https://doi.org/10.3390/molecules21091208

19. El-Hawary, S. S., Sayed, A. M., Issa, M. Y., Ebrahim, H. S., Alaaeldin, R., Elrehany, M. A., Abd El-Kadder, E. M., & Abdelmohsen, U. R. (2021). Anti-Alzheimer chemical constituents of Morus macroura Miq.: Chemical profiling, in silico and in vitro investigations. *Food & Function*, *12*(17), 8078–8089. https://doi.org/10.1039/d1fo01177d

20. Fernandes, F. H., da R Guterres, Z., Violante, I. M. P., Lopes, T. F. S., Garcez, W. S., & Garcez, F. R. (2015). Evaluation of mutagenic and antimicrobial properties of brown propolis essential oil from the Brazilian Cerrado biome. *Toxicology Reports*, *2*, 1482–1488. https://doi.org/10.1016/j.toxrep.2015.11.007

21. Fernandes, F. H., da Rosa Guterres, Z., Garcez, W. S., Lopes, S. M., Corsino, J., & Garcez, F. R. (2014). Assessment of the (anti)genotoxicity of brown propolis extracts from Brazilian Cerrado biome in a Drosophila melanogaster model. *Food Research International*, *62*, 20–26. https://doi.org/10.1016/j.foodres.2014.02.029

Revista ARACÊ
ISSN: 2358-2472

22. Gregory, J., Vengalasetti, Y. V., Bredesen, D. E., & Rao, R. V. (2021). Neuroprotective Herbs for the Management of Alzheimer's Disease. *Biomolecules*, *11*(4), 543. https://doi.org/10.3390/biom11040543

23. Greig, N. H., Lahiri, D. K., & Sambamurti, K. (2002). Butyrylcholinesterase: An important new target in Alzheimer's disease therapy. *International Psychogeriatrics*, *14 Suppl 1*, 77–91. https://doi.org/10.1017/s1041610203008676

24. Grosdidier, A., Zoete, V., & Michielin, O. (2009). Blind docking of 260 protein-ligand complexes with EADock 2.0. *Journal of Computational Chemistry*, *30*(13), 2021–2030. https://doi.org/10.1002/jcc.21202

25. Grosdidier, A., Zoete, V., & Michielin, O. (2011). Fast docking using the CHARMM force field with EADock DSS. *Journal of Computational Chemistry*, *32*(10), 2149–2159. https://doi.org/10.1002/jcc.21797

26. Gülçin, İ., Scozzafava, A., Supuran, C. T., Akıncıoğlu, H., Koksal, Z., Turkan, F., & Alwasel, S. (2016). The effect of caffeic acid phenethyl ester (CAPE) on metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione S-transferase, lactoperoxidase, and carbonic anhydrase isoenzymes I, II, IX, and XII. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *31*(6), 1095–1101. https://doi.org/10.3109/14756366.2015.1094470

27. Innok, W., Hiranrat, A., Chana, N., Rungrotmongkol, T., & Kongsune, P. (2021). In silico and in vitro anti-AChE activity investigations of constituents from Mytragyna speciosa for Alzheimer's disease treatment. *Journal of Computer-Aided Molecular Design*, *35*(3), 325–336. https://doi.org/10.1007/s10822-020-00372-4

28. Javed, M. A., Ashraf, N., Saeed Jan, M., Mahnashi, M. H., Alqahtani, Y. S., Alyami, B. A., Alqarni, A. O., Asiri, Y. I., Ikram, M., Sadiq, A., & Rashid, U. (2021). Structural Modification, In Vitro, In Vivo, Ex Vivo, and In Silico Exploration of Pyrimidine and Pyrrolidine Cores for Targeting Enzymes Associated with Neuroinflammation and Cholinergic Deficit in Alzheimer's Disease. *ACS Chemical Neuroscience*, *12*(21), 4123–4143. https://doi.org/10.1021/acschemneuro.1c00507

29. Jiang, C., Xue, J., Yuan, Y., Li, Y., Zhao, C., Jing, Q., Zhang, X., Yang, M., Han, T., Bai, J., Li, Z., Li, D., & Hua, H. (2021). Progress in structure, synthesis and biological activity of natural cephalotane diterpenoids. *Phytochemistry*, *192*, 112939. https://doi.org/10.1016/j.phytochem.2021.112939

30. Joe, E., & Ringman, J. M. (2019). Cognitive symptoms of Alzheimer's disease: Clinical management and prevention. *BMJ*, *367*, l6217. https://doi.org/10.1136/bmj.l6217

31. Li, H., Wang, X., Yu, H., Zhu, J., Jin, H., Wang, A., & Yang, Z. (2018). Combining in vitro and in silico Approaches to Find New Candidate Drugs Targeting the Pathological Proteins Related to the Alzheimer's Disease. *Current Neuropharmacology*, *16*(6), 758–768. https://doi.org/10.2174/1570159X15666171030142108

32. Lima, V. H. M. de, Almeida, K. de C. R., Alves, C. C. F., Rodrigues, M. L., Crotti, A. E. M., Souza, J. M. de, Ribeiro, A. B., Squarisi, I. S., Tavares, D. C., Martins, C. H. G., & Miranda, M. L. D. (2019). Biological properties of volatile oil from Brazilian brown propolis. *Revista Brasileira de Farmacognosia*, *29*(6), 807–810. https://doi.org/10.1016/j.bjp.2019.07.004

Revista ARACÊ
ISSN: 2358-2472

33. Lipinski, C. A. (2004). Lead- and drug-like compounds: The rule-of-five revolution. *Drug Discovery Today. Technologies*, *1*(4), 337–341. https://doi.org/10.1016/j.ddtec.2004.11.007

34. Liu, S., Cao, X.-L., Liu, G.-Q., Zhou, T., Yang, X.-L., & Ma, B.-X. (2019). The in silico and in vivo evaluation of puerarin against Alzheimer's disease. *Food & Function*, *10*(2), 799–813. https://doi.org/10.1039/c8fo01696h

35. Luo, X.-T., Wang, C.-M., Liu, Y., & Huang, Z.-G. (2015). New multifunctional melatonin-derived benzylpyridinium bromides with potent cholinergic, antioxidant, and neuroprotective properties as innovative drugs for Alzheimer's disease. *European Journal of Medicinal Chemistry*, *103*, 302–311. https://doi.org/10.1016/j.ejmech.2015.08.052

36. MacKerell, A. D., Bashford, D., Bellott, M., Dunbrack, R. L., Evanseck, J. D., Field, M. J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F. T., Mattos, C., Michnick, S., Ngo, T., Nguyen, D. T., Prodhom, B., … Karplus, M. (1998). All-atom empirical potential for molecular modeling and dynamics studies of proteins. *The Journal of Physical Chemistry. B*, *102*(18), 3586–3616. https://doi.org/10.1021/jp973084f

37. Manoharan, S., Essa, M. M., Vinoth, A., Kowsalya, R., Manimaran, A., & Selvasundaram, R. (2016). Alzheimer's Disease and Medicinal Plants: An Overview. *Advances in Neurobiology*, *12*, 95–105. https://doi.org/10.1007/978-3-319-28383-8_6

38. Mohanta, T. K., Tamboli, Y., & Zubaidha, P. K. (2014). Phytochemical and medicinal importance of Ginkgo biloba L. *Natural Product Research*, *28*(10), 746–752. https://doi.org/10.1080/14786419.2013.879303

39. Olegário, L. S., Andrade, J. K. S., Andrade, G. R. S., Denadai, M., Cavalcanti, R. L., da Silva, M. A. A. P., & Narain, N. (2019). Chemical characterization of four Brazilian brown propolis: An insight in tracking of its geographical location of production and quality control. *Food Research International (Ottawa, Ont.)*, *123*, 481–502. https://doi.org/10.1016/j.foodres.2019.04.004

40. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—A visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13), 1605–1612. https://doi.org/10.1002/jcc.20084

41. Qiao, Y., Sunada, N. K., Hatada, A. E., Lange, I., Khutsishvili, M., Alizade, V., Atha, D., Ko'omoa-Lange, D.-L., & Borris, R. P. (2019). Potential anti-neuroblastoma agents from Juniperus oblonga. *Biochemical and Biophysical Research Communications*, *516*(3), 733–738. https://doi.org/10.1016/j.bbrc.2019.06.123

42. Rao, T., Tan, Z., Peng, J., Guo, Y., Chen, Y., Zhou, H., & Ouyang, D. (2019). The pharmacogenetics of natural products: A pharmacokinetic and pharmacodynamic perspective. *Pharmacological Research*, *146*, 104283. https://doi.org/10.1016/j.phrs.2019.104283

43. Reveglia, P., Cimmino, A., Masi, M., Nocera, P., Berova, N., Ellestad, G., & Evidente, A. (2018). Pimarane diterpenes: Natural source, stereochemical configuration, and biological activity. *Chirality*, *30*(10), 1115–1134. https://doi.org/10.1002/chir.23009

Revista ARACÊ
ISSN: 2358-2472

44. Ribeiro, V. P., Arruda, C., Aldana-Mejia, J. A., Bastos, J. K., Tripathi, S. K., Khan, S. I., Khan, I. A., & Ali, Z. (2021). Phytochemical, Antiplasmodial, Cytotoxic and Antimicrobial Evaluation of a Southeast Brazilian Brown Propolis Produced by Apis mellifera Bees. *Chemistry & Biodiversity*, *18*(9), e2100288. https://doi.org/10.1002/cbdv.202100288

45. Ribeiro, V. P., Arruda, C., Mejía, J. A. A., Candido, A. C. B. B., Dos Santos, R. A., Magalhães, L. G., & Bastos, J. K. (2020). Brazilian southeast brown propolis: Gas chromatography method development for its volatile oil analysis, its antimicrobial and leishmanicidal activities evaluation. *Phytochemical Analysis: PCA*. https://doi.org/10.1002/pca.2988

46. Rodrigues, J. S. da M., & Costa, E. D. (2021). In silico ADME/T prediction of novel potential inhibitors against dengue virus Author. *Research, Society and Development*, *10*(4), Artigo 4. https://doi.org/10.33448/rsd-v10i4.14459

47. Sahoo, A. K., Dandapat, J., Dash, U. C., & Kanhar, S. (2018). Features and outcomes of drugs for combination therapy as multi-targets strategy to combat Alzheimer's disease. *Journal of Ethnopharmacology*, *215*, 42–73. https://doi.org/10.1016/j.jep.2017.12.015

48. Samadi, A., Estrada, M., Pérez, C., Rodríguez-Franco, M. I., Iriepa, I., Moraleda, I., Chioua, M., & Marco-Contelles, J. (2012). Pyridonepezils, new dual AChE inhibitors as potential drugs for the treatment of Alzheimer's disease: Synthesis, biological assessment, and molecular modeling. *European Journal of Medicinal Chemistry*, *57*, 296–301. https://doi.org/10.1016/j.ejmech.2012.09.030

49. Santos, M. F. C., Oliveira, L. C., Ribeiro, V. P., Soares, M. G., Morae, G. de O. I., Sartori, A. G. de O., Rosalen, P. L., Bastos, J. K., de Alencar, S. M., Veneziani, R. C. S., & Ambrósio, S. R. (2021). Isolation of diterpenes from Araucaria sp Brazilian brown propolis and development of a validated high-performance liquid chromatography method for its analysis. *Journal of Separation Science*, *44*(16), 3089–3097. https://doi.org/10.1002/jssc.202100374

50. Sarkar, P., Jayaraj, P., Patwardhan, K., Yeole, S., Das, S., Somaiya, Y., Desikan, R., & Thirumurugan, K. (2021). In Silico Analysis to Link Insulin Resistance, Obesity and Ageing with Alzheimer's Disease. *Journal of Molecular Neuroscience: MN*, *71*(12), 2608–2617. https://doi.org/10.1007/s12031-021-01875-x

51. Scheltens, P., De Strooper, B., Kivipelto, M., Holstege, H., Chételat, G., Teunissen, C. E., Cummings, J., & van der Flier, W. M. (2021). Alzheimer's disease. *Lancet (London, England)*, *397*(10284), 1577–1590. https://doi.org/10.1016/S0140-6736(20)32205-4

52. Shahinozzaman, M., Taira, N., Ishii, T., Halim, M. A., Hossain, M. A., & Tawata, S. (2018). Anti-Inflammatory, Anti-Diabetic, and Anti-Alzheimer's Effects of Prenylated Flavonoids from Okinawa Propolis: An Investigation by Experimental and Computational Studies. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, *23*(10), 2479. https://doi.org/10.3390/molecules23102479

53. Shapiro, D., Deering-Rice, C. E., Romero, E. G., Hughen, R. W., Light, A. R., Veranth, J. M., & Reilly, C. A. (2013). Activation of Transient Receptor Potential Ankyrin-1 (TRPA1) in Lung Cells by Wood Smoke Particulate Material. *Chemical research in toxicology*, *26*(5), 750–758. https://doi.org/10.1021/tx400024h

Revista ARACÊ
ISSN: 2358-2472

54. Shen, X., Liu, Y., Luo, X., & Yang, Z. (2019). Advances in Biosynthesis, Pharmacology, and Pharmacokinetics of Pinocembrin, a Promising Natural Small-Molecule Drug. *Molecules (Basel, Switzerland)*, *24*(12), E2323. https://doi.org/10.3390/molecules24122323

55. Thangarasu, P., Thamarai Selvi, S., & Manikandan, A. (2018). Unveiling novel 2-cyclopropyl-3-ethynyl-4-(4-fluorophenyl)quinolines as GPCR ligands via PI3-kinase/PAR-1 antagonism and platelet aggregation valuations; development of a new class of anticancer drugs with thrombolytic effects. *Bioorganic Chemistry*, *81*, 468–480. https://doi.org/10.1016/j.bioorg.2018.09.011

56. Tsui, K.-H., Wang, J.-Y., Wu, L.-S., & Chiu, C.-H. (2012). Molecular Mechanism of Isocupressic Acid Supresses MA-10 Cell Steroidogenesis. *Evidence-Based Complementary and Alternative Medicine*, *2012*, e190107. https://doi.org/10.1155/2012/190107

57. Waller, S. B., Peter, C. M., Hoffmann, J. F., Picoli, T., Osório, L. da G., Chaves, F., Zani, J. L., de Faria, R. O., de Mello, J. R. B., & Meireles, M. C. A. (2017). Chemical and cytotoxic analyses of brown Brazilian propolis (Apis mellifera) and its in vitro activity against itraconazole-resistant Sporothrix brasiliensis. *Microbial Pathogenesis*, *105*, 117–121. https://doi.org/10.1016/j.micpath.2017.02.022

58. Wang, L., Chiang, H.-C., Wu, W., Liang, B., Xie, Z., Yao, X., Ma, W., Du, S., & Zhong, Y. (2012). Epidermal growth factor receptor is a preferred target for treating Amyloid-β–induced memory loss. *Proceedings of the National Academy of Sciences*, *109*(41), 16743–16748. https://doi.org/10.1073/pnas.1208011109

59. Wang, M., Song, W.-M., Ming, C., Wang, Q., Zhou, X., Xu, P., Krek, A., Yoon, Y., Ho, L., Orr, M. E., Yuan, G.-C., & Zhang, B. (2022). Guidelines for bioinformatics of single-cell sequencing data analysis in Alzheimer's disease: Review, recommendation, implementation and application. *Molecular Neurodegeneration*, *17*(1), 17. https://doi.org/10.1186/s13024-022-00517-z

60. Wang, X., Sankarapandian, K., Cheng, Y., Woo, S. O., Kwon, H. W., Perumalsamy, H., & Ahn, Y.-J. (2016). Relationship between total phenolic contents and biological properties of propolis from 20 different regions in South Korea. *BMC Complementary and Alternative Medicine*, *16*, 65. https://doi.org/10.1186/s12906-016-1043-y

61. Wilms, W., Woźniak-Karczewska, M., Corvini, P. F.-X., & Chrzanowski, Ł. (2019). Nootropic drugs: Methylphenidate, modafinil and piracetam - Population use trends, occurrence in the environment, ecotoxicity and removal methods - A review. *Chemosphere*, *233*, 771–785. https://doi.org/10.1016/j.chemosphere.2019.06.016

62. Yan, L., He, X., Jin, Y., Wang, J., Liang, F., Pei, R., Li, P., Wang, Y., & Su, W. (2020). Modulation of the Aβ-Peptide-Aggregation Pathway by Active Compounds From Platycladus orientalis Seed Extract in Alzheimer's Disease Models. *Frontiers in Aging Neuroscience*, *12*, 207. https://doi.org/10.3389/fnagi.2020.00207

63. Yuan, H., Ma, Q., Cui, H., Liu, G., Zhao, X., Li, W., & Piao, G. (2017). How Can Synergism of Traditional Medicines Benefit from Network Pharmacology? *Molecules (Basel, Switzerland)*, *22*(7), E1135. https://doi.org/10.3390/molecules22071135