Medicinal plants of Brazil and Alzheimer's disease: Evolution in traditional use and pre-clinical studies.

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Review Article

Article Info:
Received on:16/06/2016
Published on:27/07/2016

QR Code for mobile

Literati Open Access

ABSTRACT :
In the literature, there is a growing search for new inhibitors of acetylcholinesterase (AChE) enzyme in extracts and essential oils of plants. Inhibition of AChE increases the acetylcholine concentration in the synapse, region of communication between neurons in the brain, which would decrease and retard the progression of symptoms in the treatment of Alzheimer's disease (AD). This article brings several screenings with extract and constituents isolated of Brazilian plants, as well as essential oils and their constituents as acetylcholinesterase inhibitors (AChEI), being suggested as promising for elaboration of new herbal medicines. Promising pre-clinical studies were conducted with compounds isolated from some of these Brazilian species namely *Platonia insignis* (bacurizeiro), *Citrus limon* (lemon), *Citrus sinensis* (orange), *Mangifera indica* (mango) and *Kalanchoe brasiliensis* (courama branca), for the prevention or progression of AD.

Keywords: Medicinal plants; Alzheimer's disease; Acetylcholinesterase inhibitors; preclinical studies.

INTRODUCTION:

Among the main activities for the treatment of neurodegenerative diseases, in the literature there is a growing search for new inhibitors of the enzyme acetylcholinesterase (AChE) in plant extracts, mainly involving medicinal plants already used in traditional medicine in the treatment of various diseases[1] as insomnia, amnesia , depression and anxiety or to prolong longevity, improved memory and cognitive function. [2] The investigation of the effects of active compounds extracted from plants is necessary, since they may provide new insights into the clinical treatment of neurodegenerative diseases such as Alzheimer's disease (AD). Studies suggest that AChE inhibition increases the concentration of acetylcholine at synapses, region of communication between neurons in the brain, which attenuate and slow down the progression of symptoms of AD. Some studies indicate drugs derived from natural products as inhibitors of AChE, which would make them effective in the treatment of AD. Among these drugs, stands out galanthamine, an alkaloid isolated from plants of the family Amarilidaceae which is an inhibitor, approved by the US Food and Drug Administration agency (FDA) for the treatment of AD.

Alzheimer's disease is a neurodegenerative disease, which initially reaches the memory and subsequently reasoning ability and communication.[3,4] The treatment consists in an attempt to restore the cholinergic function. [5] The brain regions most pathologically affected in AD are the hippocampus and neocortex, and these areas are associated with functions of the central nervous system of most prevalent form. [1]

The literature reports a growing number of studies which suggest that diverse behavioral effects must be associated with different hippocampal subregions. [1] The dorsal hippocampus, for example, have a preferential role in certain types of learning and memory, which are markedly affected in AD. [6]

In this review we focus on plants of Brazilian fauna as inhibitors of AChE and preclinical tests already carried out. Some considerations about the AD, and the importance of supplementation with antioxidants in the treatment of neurodegenerative diseases are also reported in this work. Many of the listed species and their isolated compounds, being inhibiting AChE, appear as promising for the preparation of herbal medicines for the treatment of AD. We recorded some fruit and some of its chemical constituents that have antioxidant and inhibitory actions of AChE, which may be useful in treating this disease.

2. Inhibitory Brazilian plants of the enzyme acetylcholinesterase (AChE)

Some studies indicate drugs derived from natural products as potential inhibitors of AChE enzyme, rendering them effective in the treatment of AD. Among these drugs, we

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Conflict of interest: Authors reported none

have galantamine **(I)** (Figure 3), an alkaloid isolated from plants of the family Amarilidaceae, which is an inhibitor of AChE, as approved by the FDA for the treatment of disease. It is an inhibitor of AChE long-acting, selective, reversible, competitive, which produces beneficial effects even after the end of treatment. [7] Galanthamine is considered the drug of most effective action and that causes fewer side effects. Tacrine **(II)** (Cognex^{Ò}) has hepatotoxic effect; rivastigmine **(III)** (Exelon^{Ò}) causes undesirable side effects, and physostigmine **(IV)** (Synapton^{Ò}) is efficient only moderate in humans. [8] Therefore, it is very important to search for new inhibitors, possibly present natural products that may have few or no side effects and good level of efficacy in the treatment of AD.

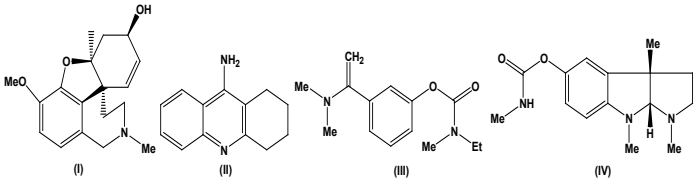

Figure 1. *AChE inhibitors marketed: I (galantamine), II (tacrine), III (rivastigmine) and IV (physostigmine)*

The search constituents of medicinal plants that inhibit AChE enzyme through a "screening" is presented as a viable and promising alternative for the development of new drugs for the treatment of AD. The screening performed by Feitosa et al [9] revealed several species of the Brazilian fauna candidate to inhibit the AChE enzyme (Table 1). The search for these inhibitors is usually performed by Ellman's colorimetric assay [10] modified by Ingkaninan [11].

The analysis of the species tested proved medicinal plants very promising for isolation and characterization of AChE inhibitor compounds. [9] The plants analyzed were collected in Center of Aromatic Plants and Medical (NUPLAM), Federal University of Piauí, Campus of Teresina-PI. The herbarium specimens of plants are deposited in the herbarium Graziella Barroso in Teresina-PI. 300.0 g of dried leaves of species were ground and subjected to extraction with hexane, ethyl acetate and methanol, respectively. For some species, tests with latex, oil or almond were performed. Solutions containing 10.0 mg mL^{-1} of each extract were used in qualitative test of Ellman with the AChE enzyme [10].**Table 1.** R*esults of inhibition of the extracts in TLC (AcOEt) and (MeOH) of species undergo a screening* [9]

Family	***Name of the specie*** (common name)	***parts used***			***Test of AChE in TLC***	
					AcOEt	***MeOH***
Acanthaceae	*Justicia pectoralis* (chamba, anador)	*leaves*	-	-	FP	P
	Myracrodruon urundeuva (mastic)	*leaves*	-	-	P	P/FP
	Anacardium occidentale L. (cashew tree)	*leaves*	LCC	-	-	-
	Spondias mombim(cajazeira)	*leaves*	-	-	FP	P
Anacardiaceae						
Asteraceae	*Acmella uliginosa* (watercress brave)		-	-	FP	FP
	Athemisia absinthium L. (wormwood)	*leaves*		-	FP	FP
	Altemanthera brasiliana L.(terramycin)	*leaves*	-	-	P	P
	Pfafia glomerata (Brasilian ginseng)	-	-		P/FP	FP
	Gomphrena globosa (perpetual)		-	-	FP	FP
Amaranthaceae						
Bignoniaceae	*Arrabidaea chica* Verlot (crajiru)	*leaves*	-	-	P	P
Brassicaceae	*Lepidium sativum* (cress)	*leaves*	-	-	FP	P
	Kalanchoe pinnata (red courama)	*leaves*	-	-	FP	P
	Kalanchoe gastonis (courama long)	*leaves*	-	-	FP	FP
Crassulaceae						
Equissetácea	*Equisetum arvense* (mackerel)	*leaves*	Latex	-	FP	P
Euphorbiaceae	*Euphorbia tirucalli* L. (avelos, naked dog)	*leaves*	Latex	-	P/FP	P/FP
Fabaceae	*Bauhinia forficata* (cow paw)	*leaves*	-	-	P	FP
	Copaifera multijuga (copaiba)	-	Oil	-	-	-
Gramineaceae	*Cimbopogom nardus* (citronella)	*leaves*	-	-	FP	P/FP
Iridaceae	*Eleuthera plicata*	*leaves*	*Almonds*	-	FP	FP
Labiatae	*Ocimum gratissimum* (basil)	*leaves*	-	-	P/FP	FP
	Plectranthus barbatus (false boldo)	*leaves*	-	-	P	P
Lamiaceae	*Plectranthus amboinicus* (basil)	*leaves*	-	-	P	P
	Mentha villosa (mint)	*leaves*	-	-	FP	P/FP
	Mentha pipperate (mint)	*leaves*	-	-	P	FP
Leguminosae	*Cenostigma ntacrophyllum* (becard)	*leaves*	-		P	P
Liliaceae	*Aloe Vera* L. (aloe vera)	*leaves*	Latex	-	FP	P
Moringaceae	*Moringa oleifera* (white acacia)	*leaves*	-	-	P	P
Meliaceae	*Azadiractha indica* (neem)	*leaves*	-	-	FP	P
Myrtaceae	*Eugenia uniflora* (pitangueira)	*leaves*	-	-	FP	FP
Schrophulariaceae	*Capraria biflora* L. (tea land)	*leaves*	-	-	P/FP	FP
	Lippia sidoides (rosemary pepper)	*leaves*	-	-	P	FP
Verbenaceae	*Lippia geminata* (lemon balm)	*leaves*	-	-	P/FP	FP
	Vitex agnus (wild pepper)	*leaves*	-	-	FP	FP
Vitaceae	*Cissus sicyoides* (plant insulin)	*leaves*	-	-	P	FP

Note. Positive-P, Negative-N, False-Positive-FP. Thin Layer Chromatography-TLC, ethyl acetate-EtOAc and methanol-MeOH. LCC-liquid of cashew nut.

The tests of the AChE enzyme activity with crude oil of species *Copaifera multijuga* Hayne was tested in three concentrations (5 $mg.mL^{-1}$, 10 $mg.mL^{-1}$ and 15 $mg.mL^{-1}$) and was shown to be active at concentrations 5 and 10 $mg.mL^{-1}$.

Medicinal plants (Table 1) grouped by family were subjected to false-positive tests to verify that the positive outcome of these plants in chromatography test thin layer (TLC) with the AChE enzyme are indeed inhibition of this enzyme or just a chemical reaction between 5,5'-dithiobis acid [2-nitrobenzoic acid] (DTNB) and acetylthiocholine iodide (ATCI). Of the species tested only the ethyl acetate extract of *Azadiractha indica* (neem), *Plectranthus barbatus* (boldo) and *Lippia sidoides* (rosemary pepper) were negative.

In this screening, quantitative assays of enzyme inhibition in microplate was performed using as standard physostigmine. In the assay microplate the specie *Cenostigma macrophyllum* Tull. Var. (becard) present that greater percentage of

inhibition, approximately 45%. The *Senna siamea* (Lam.) Irwin *et* Barneby (cassia-of-siam) and *Justicia pectoralis* Jacq. Var. stenophylla Leonard (anador) species showed the most promising results with 28% and 29% inhibition, respectively.

The findings indicated that the species *C. macrophyllum* Tul. var. acuminata Teles Freire (becard), *S. siamea* (Lam.) Irwin et Barneby and *J. pectoralis* Jacq. Var. stenophylla Leonard (Chambá; Anador) showed more promising results in the selection of plants, since they showed positive results on TLC opposite the AChE enzyme and a higher percentage of inhibition cromatoplaca (45%, 28% and 29%), respectively. The known species as becard (*C. macrophyllum*) proved the most promising in search of AChE inhibitors. [9]

In another screening conducted with plants collected in Fortaleza-CE, species were selected belonging to the family: Leguminosae, Crassulaceae, Euphorbiaceae, Malvaceae, Moraceae, Nyctaginaceae and Rutaceae.[35] This selection were collected 18 plants in the Garden of Medicinal Plants Francisco José de Abreu Matos, at the Federal University of Ceará. Prepared extracts ethyl acetate (EtOAc) and methanol (MeOH) of leaves, flowers and stems of plants and tests were conducted AChE enzyme inhibition assays (Table 2). [36] The values of median inhibitory concentration (IC_{50}) of the enzyme AChE reported to the extracts of these species were: *Ipomoea asarifolia* (IC_{50} = 0.12 mg mL^{-1}.), *Jatropha curcas* (IC_{50} = 0.25 mg mL^{-1}.), *Jatropha gossypiifolia* (IC_{50} = 0.05 mg.mL^{-1}), *Kalanchoe brasiliensis* (IC_{50} = 0.16 mg.mL^{-1}) and *Senna alata* (IC_{50} = 0.08 mg.mL^{-1}). These results were significant compared to standard positive, galantamine (IC_{50} = 0,37x10^{-3} mg.mL^{-1}), being crude extracts.

Table 2: *Results of inhibition of medicinal plants in microplate in a phytochemical screening* [13]

Family	*Plant (common name)*	*Parts used*			*Microplate (%) (2 mg mL^{-1})*	
					AcOEt	**MeOH**
Convolvulaceae	*Ipomoea asarifolia* (parsley)	f	-	-	100	100
		-	fl	-	29	49
	Ipomoea batatas Poir. (sweet potato)	f	-	-	89	86
Crassulaceae	*Kalanchoe brasiliensis* Pers. (white courama)	f	-	-	100	100
	Kalanchoe pinnata Pers. (red courama)	f	-	-	100	15
	Kalanchoe gastonis- (long courama)	f	-	-	82	2
Euphorbiaceae	*Phyllanthus amarus* Schum. *et* Torn. (breaking stone)	f	-	-	69	32
		-	fl	-	100	11
	Jatropha curcas L. (Jatropha)	-	-	t	100	78
		f	-	-	61	100
	Jatropha gossypiifolia L. (purple pinion)	f	-	-	71	100
	Jatropha pohliana Muell. Arg. (brave pinion)	f			82	49
Leguminosae	*Caesalpinia ferrea* Mart. *et* Tul. (pau ferro)	f	-	-	37	71
		-	-	t	13	54
	Senna reticulata (L.) Irw. *et* Barn. (big mangerioba)	f	-	-	100	43
	Senna alata (L.) Roxburgh. (mangerioba)	f	-	-	100	100
	Cassia fistula L. (canephori)	f	-	-	86	100
		-	fl	-	32	100
	Leucaena leucocephala (Lamk.) Wit. (leucaena)	f	-	-	14	69
Malvaceae	*Gossypium herbaceum* L. (cotton)	f	-	-	37	73
Moraceae	*Ficus benjamina L.* (ficus)	f	-	-	96	100
Nyctaginaceae	*Bougainvillea glabra* Choisy (Boungainvile)	f	-	-	100	56
		-	fl	-	32	0
Rutaceae	*Citrus limmonia* Osbeck (lemon tree)	f	-	-	89	100

Note: Assays to 25 µL, tested of 1mg to 5 mg in 1 mL of methanol. f = leaves, fl = flowers, t = stalks, P = positive, N = Negative.

In a study by Feitosa, [36] monitoring inhibition of AChE enzyme with *Anacardium occidentale* L. species (Anacardaceae), known as cashew tree, a species that produces a fruit that is the cashew nuts, provided the monoene and diene anacardic acids. In test of inhibition these acids proved to be active for the inhibition of AChE enzyme in thin-layer chromatography (TLC).[13] The mesocarp of this fruit, then extracted liquid, commercially called liquid cashew nuts (LCN), containing

classes of compounds also present in the fruit and leaves extract of *Ginko biloba*, the ginkgolicos acids (Sin. anacardic acids).[14]

A screening done by Trevisan et al,[15] also suggests that there are many other medicinal plants in Brazil candidates for the isolation of AChE inhibitors (Table 3).

Table 3. *Species subject to the enzyme AChE inhibition test in screening*[15]

Medicinal plants (common name)	Medicinal plants (common name)
Amburana cearenses (tonka bean)	*Mormodica charantia* (São Caetano melons)
Anacardium occidentale (cashew)	*Paullinia cupana* (guarana)
Auxema glazio*viana* (white stick)	*Philodendron Imbé* (clove buzzard)
Bauhinia Cheilantha (mororó)	*Plathymenia reticulata* (amarelinho)
Bowdicha virigilioide (black sucupira)	*Plathymiscium floribundum* (rosewood pink)
Cecropia pachystachya (umbaúba)	*Protium heptahyllum* (almecegueira)
Cucumis anguria (gherkin)	*Pterodon polygalaeflorus* (white sucupira)
Cordia piauhiensis	*Simarouba versicolor* (pau-paradise)
Croton urucunama (urucuana)	*Solanum asperum* (itches-itches)
Dalechampia fernandesii	*Triphasisa trifólia* (lemon China)
Egletes viscosa (Macela)	*Vanillosmopsis arbórea* (sconce)
Lippia alba (lemongrass)	*Verbesina diversifolia* (camara)
Lippia sidoides (rosemary pepper)	*Vitex agnus castus* (chastity tree)
Lonchocarpus sericeus (ingazeira-peeped)	*Macrosiphonia velame* (canopy)
Mimosa acustipula (black Jurema)	

Adapted from Trevisan et al.[38]

In this search performed by Trevisan et al,[15] the species were collected in Fortaleza-CE and results disclosed were very promising. Among the most promising species *Paullinia cupana* (guarana), presented 65% of AChE inhibition in microplate for ethanolic extract of leaves, moreover presented positive result in TLC for Elmman's assay.[10]

Caffeine, one of the inhibitors frequently used as positive standard inTLC is a white and solid alkaloid that can be obtained from guarana, coffee, tea, yerba mate, among others. In unpublished research carried out by Mohamed et al,[16] this alkaloid inhibited the enzyme acetylcholinesterase and butilcolinesterase enzymes.

The species *Lippia sidoides* (rosemary pepper) showed inhibition of AChE from 7% (ethyl acetate extract of the bark) to 77% (ethanol extract of the leaves). For the species *Plathymiscium floribundum* (Table 3) was conducted tests with ethanolic extract of heartwood and that they had a 72% inhibition in the microplate and test positive in TLC to inhibition of AChE. *Plathymenia reticulata* (stem bark); *Solanum asperum* (leaves) and *Triphasia trifolia* (leaves) were presented inhibition of 88%; 60% and 53% in microplate, respectively. The species *P. reticulata*, *S. asperum* and *T. trifolia* are popularly known by the names: yellowing, scratch-scratch lemon and China, respectively.

Another species with promising results in the screening performed by Trevisan et al [15] was *Amburana cearensis* (tonka bean), which extract inhibited AChE enzyme in 100% in microplate and demonstrated positive result for inhibition of AChE in TLC.

Marques *et al,* [1] evaluated the acetylcholinesterase activity of the ethanol extract of flowers (EEF) and the enriched fraction of flavonoids isolated of species *Bellis perennis* L., known as common daisy. In *in vitro* studies, there was an inhibition of AChE activity 83.85 and 17.22 when used rivastigmine (Exelon) as positive control at concentrations of 0.2 and 0.0125%, respectively. The enriched fraction of flavonoids isolated from the FES, in concentrations of 0.0125; 0.00652; 0.003125 and 0.001563% produced an inhibition of 64.30; 55.83; 43.87 and 38.13% in the AChE activity, respectively. Based on these results, it was also determined the inhibitory concentration (IC_{50}), corresponding to 3.1 μmol L^{-1}, ranging from 1.7 to 6.1 μmol L^{-1} with 95% confidence interval. Complementing the analysis of the inhibitory effects on the activity of AChE *in vivo*, this study was observed a decrease of 81, 83 and 82% after treatment with doses of 50 mg. Kg^{-1} (1.91 ± 0.25; p <0.05), 100 mg. Kg^{-1} (1.66 ± 0.21; p <0.05) and 150 mg kg^{-1} (1.79 ± 0 13, p <0.05) of EPS compared to the negative controls (10.03 ± 0.16; p <0.05), respectively. Likewise, compared to the positive control group treated with rivastigmine (5.69 ± 1.20) showed a reduction of 67, 71 and 69% of AChE activity in mice treated with doses of 50 mg kg^{-1} (1.91 ± 0.25; p <0.05), 100 mg.Kg^{-1} (1.66 ± 0.21; p <0.05) and 150 mg kg-1 (1.79 ± 0 13, p <0.05) FES, respectively. Furthermore, a decrease of 91% was observed in the AChE activity in mice treated with 10 mg .Kg^{-1} (0.89 ± 0.21) of the enriched fraction

of flavonoids of FES isolated in *B. perennis* compared to the negative controls (10.03 ± 0.16; p <0.05). In comparison to the positive control group treated with rivastigmine (5.69 ± 1.20) there was a 84.4% reduction in AChE activity in mice treated with 10 mg kg^{-1} (0.89 ± 0.21, p <0.05) the isolated compound. The enriched fraction of flavonoids isolated from the FES also produced a reduction of 53.4; 46.4 and 50.3% in the AChE activity in mice treated with 10 mg kg^{-1} (0.89 ± 0.21) compared to the groups treated with the doses of 50 mg kg^{-1} (1.91 ± 0.25, p <0.05), 100mg kg^{-1} (1.66 ± 0.21; p <0.05) and 150 mg kg^{-1} (1.79 ± 0.13; p <0.05) of FES from *B. perennis*, respectively.

Another species with isolated constituents with very promising results on AChE was the *Kalanchoe brasiliensis* plant (white courama), Brazilian medicinal species of Crassulaeae family, used in folk medicine in the treatment of chronic inflammatory diseases such as rheumatism. Scientific studies have shown analgesic and anticonvulsant effects of *K. brasiliensis* stem extracts.[17] The hydroalcoholic extract of fresh leaves of white courama showed inhibitory property of acetylcholinesterase in rectus abdominis isolated frogs in experiments by Fonteles et al. [18] In these experiments, the inhibitory effect of d-tubocurarine in contractile responses induced by acetylcholine was effectively blocked by the extract, the same occurring with prostigmine, a well-known anticholinesterase. In a monitored study done by Feitosa[13] obtained in the isolation of active glycosidic flavonoids: 8-metoxiquercetina 3-*O*-a-L-raminopiranosil-7-*O*-a-L-raminopiranosídeo (**1**) and 8-metoxicanferol 3-*O*-raminosídeo-7-O-a-L-raminopiranosídeo (**2**) (**Figure 2**), these novel compounds in the genus *Kalanchoe* which could be responsible for the inhibitory effect of cholinesterase, which was detected in the phytochemical approach of hydroalcoholic extract of this species .

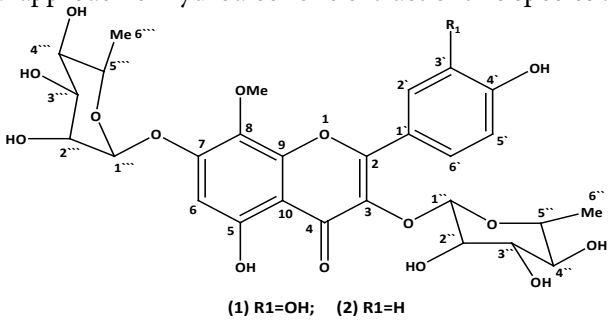

Figure 2. *Structure of the inhibitors alkaloids of AChE isolated from K. brasiliensis Inhibitors acetylcholinesterase* [13]

The importance of cholinergic function in learning and memory processes is known since the early 70's,[19] and research work about the importance of the cholinergic system in AD demonstrated several characteristicst, such as the decrease in the concentration of choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine (ACh) in the cortex and hippocampus, as well as a variable decrease of cholinergic neurons in the basal nucleus of Meynert.[20]

It is believed that inhibition of the enzyme acetylcholinesterase (AChE) increase the concentration of acetylcholine in the synapse, which would decrease or retard the progression of symptoms of AD. In cholinergic hypothesis, people suffering from AD have low levels of acetylcholine, an important neurotransmitter. Inhibitors of AChE enzyme slows metabolic degradation of acetylcholine, thereby optimizing availability of the substrate for the communication between cells. This helps to delay the progression of cognitive impairment and can be effective for some patients the initial and middle stages of the disease.[10,11] There are now four drugs belonging to this class approved by the FDA, to know: tacrine, donepezil, rivastigmine and galantamine. Marketed inhibitors usually have detrimental effects. In this respect it is of great value to search for new inhibitors present in natural products that may have fewer side effects.

The causes of the development of AD are not fully understood, but the information about certain changes in the brain tissue are characteristic of the disease. Among these changes, they can be identified: formation of extracellular amyloid plaques, which are extracellular deposits of amorphous b-amyloid protein and microtubule, formation of microtubules and intraneuronal neurofibrillary tangles. In normal brain, these configurations present in minor amounts.[5,8,9-10] The enzymes butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) are present in the brain and are found in neuritic plaques and neurofibrillary tangles. To the extent that inhibition of acetylcholinesterase is modified by the deposition of b-amyloid protein, this method is considered a key component for understanding the pathophysiology of AD.[21]

Assays of plant extracts are widely used to test the inhibitory activity of the enzyme acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The neurotransmitter acetylcholine is inhibited AChE first and then by BuChE. The measure of AChE activity can be obtained by an easy and rapid assay based on the method of Ellman,[33] modified by Rhee et al [22] in this procedure the reagents used are 5,5'-dithiobis [2- nitrobenzoic acid] (DTNB) and acetylthiocholine iodide (ATCI).

2.1 Antioxidants essential oils and AChE inhibitors and oxidative stress

Essential oils (EO) are volatile oils obtained from aromatic plants that can be extracted from different techniques: hydrodistillation, drag by supercritical CO_2, distillation by steam distillation, "enfleurage", among others. EO has been investigated for promising activities: antioxidant, anti-inflammatory, analgesic and acetylcholinesterase. The chemical

composition of EO varies considerably from species to species, depending on climatic parameters and agronomic factors such as fertilization, irrigation, and especially the development phase in the plant during harvest. The monoterpenes and sesquiterpenes compounds are major constituents found in the essential oils.[23]

Plants that have shown favorable effects on the cognitive disorders, including anticholinesterasic, antiinflammatory and antioxidant activities or other pharmacological activities are of potential interest for clinical use in the treatment of AD. Plants that affect cholinergic function in the central nervous system (CNS) are particularly relevant in the treatment of AD.[2.24] Pharmacological investigation of natural products having activity on the central nervous system (CNS) has been aiding understanding the neurochemical bases many diseases.

Oxidative stress is a major mechanism underlying the development of various neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's and amyotrophic lateral sclerosis).[25] These diseases are the main characteristics of progressive and irreversible loss of structure and/or function of neurons various brain regions. The brain region and types of affected neuronal cells develop a set of behavioral characteristics and cognitive and/or specific motor for each disease as cortical neurons in AD.[26] The chemical substances which possess one or more unpaired electrons are chemically known as of free radicals, which have great facility for donating their electrons to other molecules causing chain reactions and consequently various oxidative damage. Free radicals and related molecules can be classified as reactive oxygen species (ROS) and reactive nitrogen species derived (RNSs). Moreover, many of these reactive entities are produced during normal metabolism in biological systems, which are offset by enzymatic and non-enzymatic cellular antioxidant mechanisms.[27] The *in vitro* evaluation of parameters related of the oxidative stress have provided a preliminary view on the pathogenesis of many neurodegenerative diseases. In this way, it has demonstrated the mechanism of action of ROS/RNSs these diseases by direct or indirect detection by various experimental procedures *in vitro*.[25]

A study done by Sa et al. .[28] about the essential oil of fresh leaves (OEF) from the species *Citrus sinensis* (L.) extracted by hydrodistillation by an extractor type Clenvenger. The EOF analysis of this species by GC-MS resulted in the identification of the mixing constituents: limonene (20.14%), citronellol (30.42%), geranial (31.42%), myrcene (0.64%) trans-β-ocimene (0.73%), linalool (2.58%), citronellal (1.23%), neral (1.71%) and β-caryophyllene (2.04%). The chemical structures of these constituents can be seen in Figure 3.[28]

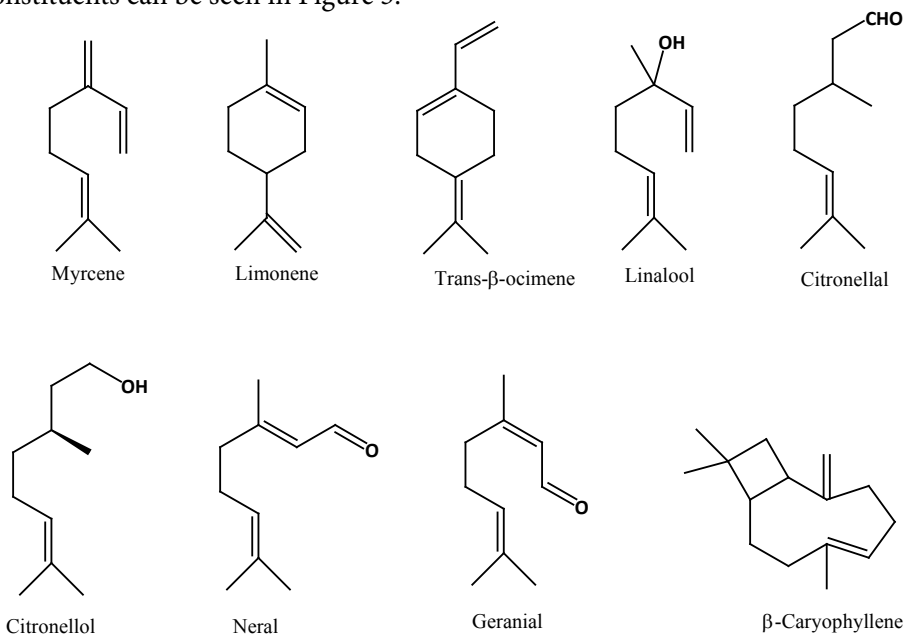

Figure 3. *Chemical structure of the constituents identified in EO of C. sinensis (orange)*

The effects of oral administration acute with repeated doses of EOF of *C. sinenses* (orange) were investigated by evaluating the biochemical and hematological parameters in mice *Swiss* adult males. It was also evaluated the *in vitro* activity and *in vivo* AChE enzyme and antioxidant potential in the hippocampus of mice. The mice were treated for 30 days with the EOF of *C. sinenses* at doses of 50, 100 and 200 mg.Kg^{-1}. The biochemical and hematological parameters EOF of *C. sinenses* produced no toxic effects on Swiss mice only there was a decrease in triglyceride levels. Evaluation studies of antioxidant activity *in vivo* with the species *C. sinensis* (L.) Osbeck (orange) suggested that there was a significant 20% reduction after treatment with a dose of essential oil of 150 mg.Kg^{-1} at the level lipid peroxidation, reducing oxidative stress and formation of nitrite, suggesting that there was a significant decrease in all groups of animals (mice) treated, providing protection against brain damage to permanent neurochemical changes. Results of acetylcholinesterase activity EOF of *C. sinensis*, suggests that there was a significant decrease in hippocampal region with an IC_{50} = 0.07 μg mL^{-1} whereas for the standard (neostigmine) was obtained value of IC_{50} = 1.87 μg.mL^{-1}. Pharmacological studies with essential oil of *C.sinensis* revealed a significant decrease of 73, 83 and 76% of AChE activity in the hippocampus of experiments in animals treated for 30 days with doses of 50, 100 and 200 mg.kg^{-1}, respectively. In this study it was observed that the value

of the concentration inhibition of EO of *C.sinensis* L. (orange) was IC_{50} = 0.07 μg.mL^{-1} while the inhibition percentage of the positive control, neostigmine was IC_{50} = 1.87 μg.mL^{-1}.[28]

Other highly relevant studies were published in the literature with essential oils of other species. The acetylcholinesterase activity of the essential oil *in vitro* of species *Eucalyptus camaldulensis* Dehnh (eucalyptus) and *Ocimum canum* Sims. (Basil) are very promising compared the species *Salvia lavandulaefolia* Vahl. (Sage) and *Rosmarinus officinalis* L. (rosemary) that demonstrated some valuable therapeutic effects. Studies of essential oils of *S. lavandulaefolia* Vahl. (Sage), suggest that the results are relevant in the treatment of Alzheimer's dementia [29] which showed IC_{50} = 50 μg.mL^{-1}, [30] while the species *A. officinalis* (rosemary) [31] an IC_{50} = 70 ug.mL^{-1} improving the performance and overall quality of memory in healthy adults.[32] The results analyzed in the the studies with EO of *C. sinensis* showed that after treatment in mice, there was a significant decrease in activity AChE in the hippocampus, which justifies the search for inhibitors of this species.

In several regions of Brazil the essential oils of other species have been analyzed for their antioxidant and anticholinesterase potential. The results were very favorable for the species *Piper hispidum* (matico), *Piper aleyreanum* (paninixpu) and *Piper anonifolium* (long pepper), collected in the National Forest of Carajés in Pará, where their essential oils were obtained by hydrodistillation and analyzed by GC and GC MS.[33]

They identified 87 constituents (monoterpenes and sesquiterpenes) in oils of three species of *Piper*. The constituents identified were the sesquiterpenes: selin-11-en-4-α-ol, β-elemene, β-selinene, α-selinene, bicyclogermacrene, β-caryophyllene, α-humuleno, and δ-elemene. The results analyzed for anticholinesterase activity of essential oils of the species *P. anonifolium* and *P. hispidum* were motivators as the results were more potent for these species when compared to one of the drugs used in the treatment of AD, physostigmine, used in the experiment as standard positive.[33] These results suggest that the *Piper* oils will can be used as medicaments in neurological disorders.

The lead compounds identified in the oils of some AChE inhibitor species (Figure 4) were 1,8-cineole (33.9%), α-pinene (12.5%), p-cymene (12.3%) limonene (11.5%) to *Eucalyptus camaldulensis* Dehnh (eucalyptus); 1,8-cineole (59.9%), camphor (8.1%) to *Ocimum canum* Sims. ("alfavaca") and linalool (48.7%), eugenol (27.5%) to *Ocimum basilicum* L. to (basil). Except eugenol compound, all other compounds have been previously found to inhibit AChE individually, and 1,8-cineole the most potent followed by α-pinene, camphor and linalool.[30.34] The mixtures of compounds 1,8-cineole and α-pinene showed a synergistic effect, while the mixture of the compound 1,8-cineole and camphor antagonized anticholinesterase activity.[30]

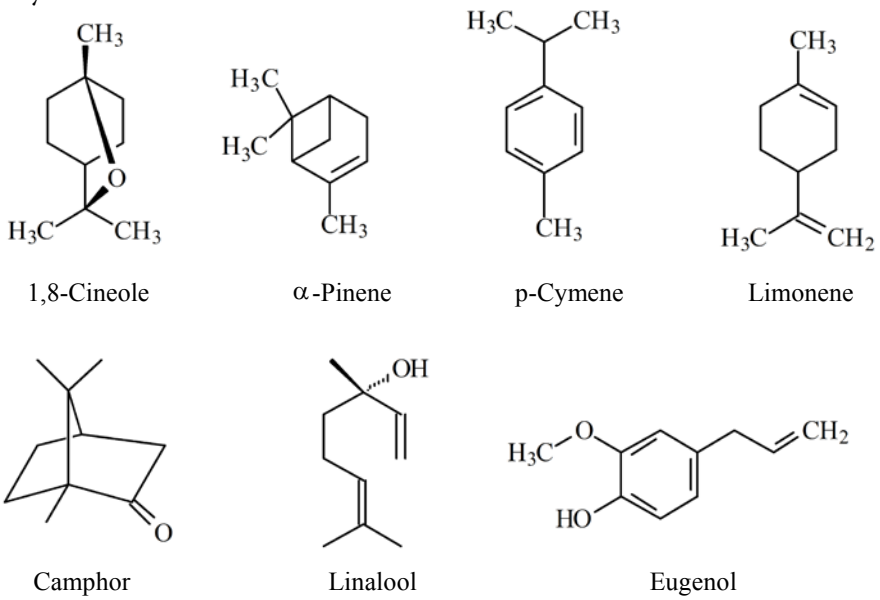

Figure 4. *Identified compounds in the essential oils of some AChE inhibitory species*

2.2 Antioxidants compounds in fruits and Alzheimer's disease

Antioxidants can act in different ways in the treatment of AD to contribute to the integrity of neuronal tissue: inhibition of the activity of superoxide dismutase and monoamine oxidase enzimes, which contribute to generation of free radicals in the brain and body; kidnapping of free radicals, which could cause damage to neurons and consequently delay the changes associated with aging of the brain; reducing the release of arachidonic acid, a toxic product of lipid metabolism, which appears in the brain after ischemic episode.[35]

Scientific studies have shown that some fruits namely: lemon (*Citrus limon*), orange (*Citrus sinensis*), mango (*Mangifera indica*), cashew (*Annacardium ocidentale*) jenipapo (*G. americana* L.), hog plum (*Spondia purpurea* L.) umbu (*Spondia tuberosa* L.) and and soursop (*Annona crasiflora* Mart.) showed promising acetylcholinesterase activity. Potent antioxidant activity is also reported for most of these fruits.

Among the plants introduced in Brazil with great economic importance, there is the *Mangifera,* name common hose, which produces sleeves palatable. The leaves of *Mangifera indica* L. (hose) are important as a source of phenolic compounds,

especially mangiferin, a C-glycosylated xanthone with aspect of yellow solid called: 2-C-β-D-glucopyranosyl-1,3,6,7--tetrahidroxi-xanthone (**Figure 5**), which has promising properties: antidiabetic, antioxidant, anti-inflammatory and anticholinesterase. Important hepatoprotective effect of this substance was demonstrated by Yoshikawa et al[36] where this compound attenuated the liver injury in mice exposed to carbon tetrachloride by neutralization of trimethyl radicals, resulting in reduced plasma levels of inflammatory enzymes glutamic oxaloacetic transaminase (GOT) and transaminase glutamic pyruvic (TGP).[37] In these studies mangiferin (50 mg. kg^{-1}, p.o) was administered once a day for 7 days and prevented oxidative damage of liver in mice subjected to toxicant 13- acetate of 12-O- tetradecanoylphorbol, reducing the loss of sulfhydryl groups in homogenates, lipid peroxidation in microsomes and mitochondria and DNA fragmentation in liver tissues.[38] The hepatoprotective action of mangiferin is of great importance due to reports of some drugs such as tacrine, which is used in the treatment of AD present hepatotoxic action.

Biradar et al.[62] conducted preclinical studies with animals where this research neuropharmacological activities of mangiferin were analyzed by assessing effects on memory and anticholinesterase *in vivo* in mice. The mangiferin was tested at doses of 40, 20 and 10 mg. Kg^{-1}, the results confirmed that animals treated with this compound significantly improved the ability of learning and memory retention in passive avoidance test and elevated plus maze, pharmacological tests used to evaluate behavior and memory. Pretreatment with mangiferin increased acetylcholine by blocking the action of acetylcholinesterase in whole brain, restored lipid peroxidation and reduced glutathione, due to the action of scopolamine used in the experiment and natural aging. The data presented in this study suggest using this compound isolated from a medicinal plant well known in the treatment of Alzheimer's disease.[39]

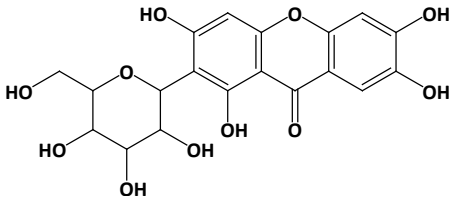

Figure 5. Structure of *mangiferin*

For species (*Anacardium occidentale*), cashew tree, a species of the genus *Anacardium* and family Anacardiaceae, there are different designations, in different languages, since this plant is found in several countries. In Portuguese-speaking countries, the terms related to this plant are: cashew, cashew tree, cashew foot, brown-of-cashew apple-of-cashew, among others. The liquid of bark of cashew nut (LCN) is referred to in the literature by the acronym CNSL (Cashew Nut Shell Liquid). The cashew tree has several compounds recorded in literatura with important activities related to the treatment of AD.

There are reports in the literature of volatile compounds in the essential oil of leaves, flowers and fruit of the cashew tree. The compounds (*E*)-*b*-ocimeno, a-copaeno and d-cadineno were identified in leaves of red cashew. The compounds: palmitic acid; oleic acid; furfural; 4-hydroxydodecanoic acid and (*E*)-hex-2-enal; (*Z*)-hex-3-enol and hexadecanol are the main components of the essential oil of leaves and fruits of yellow cashews. Major constituents, as b-caryophyllene, methyl salicylate and tiglate benzyl, are cited as present in red flowers of red cashew.[40]

The composition of the main components of natural LCN are anacardic acids, "cardanóis" and "cardóis". The anti-acne activity, bactericidal, antiseptic, antitumor, fungicide, molluscicide, pesticide, nematicide, among others, are cited for anacardic acids. The cashew tree produces a fruit widely known as cashew nut.[64] In the studies by Feitosa (2005)[13] the LCN provided the anacardic acids monoene and diene which have: positive results for inhibition of AChE in TLC; larvicidal activity against *Aedes aegypti* in 15 ppm concentration and antioxidant activity IC_{50} 1.52 mM and 1.81 mM, respectively. The LCN contains substances of the same class of compounds present in the extract of the fruit and leaves of *G. biloba*, the ginkgolicos acids (Sin. anacardic acids). *G. biloba* is a plant used in Indian and Chinese traditional medicine, which studies have shown the existence of relevant pharmacological activities in the treatment of cognitive disorders. Because of this property, *G. biloba* is indicated for therapeutic use against cognitive disorders caused by diseases such as AD.[13]. Carotenoids, such as b-carotene, b-cryptoxanthin and ascorbic acid (vitamin C), powerful antioxidants, are recorded as present in the commercial juice of cashews.[41] For the flavanone naringenin, the antioxidant activity, anti-inflammatory, fungicide and bactericide are reported. The prunin-6-O-*p*-coumarate substance was isolated from liquid of cashew nut and shows antioxidant activity, anti-inflammatory and bactericidal. [42] The monoterpene limonene is reported as antitumor agent, insecticide, herbicide and also AChE inhibitor. [43]

The triterpenes b- amyrin and lupeol were recorded, steroids b-sitosterol and stigmasterol, catechin and epicatechin, the liquid of film of immature cashew nut.[44]

Another species with very promising results for the production of drugs against AD was bacuri, fruit of the species *Platonia insignis* Mart., belonging to the Clusiaceae family and *Platonia* gender. The Clusiaceae family consists of 1,000 species and 47 genera distributed in tropical and subtropical regions of the world. It is also a genus found in temperate regions. In nine of these, 90 species have edible fruits. The term *Platonia* is a tribute to Plato, Greek philosopher, and *insignis* means remarkable, distinguished, important, big, one that draws attention, in reference to the size and usefulness of the plant.[45,46]

Platonia insignis ("bacurizeiro") is a fruit tree species and timber, it originates in the western Brazilian Amazon, in the state of Pará and is found in all states of the North Region of Brazil and Mato Grosso, Maranhao and Piaui. It is also found in Guyana, Peru, Bolivia, Colombia and Ecuador. It has economic importance in the states of Pará, Maranhão, Tocantins and Piaui in areas of secondary vegetation.[45,47]

The "fat" extracted from *P. insignis* seeds revealed potential healing action to accelerate the healing of skin wounds in rats. The triglycerides, 1,3-distearyl-2-oleoyl glycerol (TG1) (Figure 6), was isolated from the hexane extract of "fat" the bacuri.[48] In preclinical experiments following the method of Ellman[10] , TG1 inhibit the enzyme acetylcholinesterase *in vivo* and *in vitro*.[49.50]

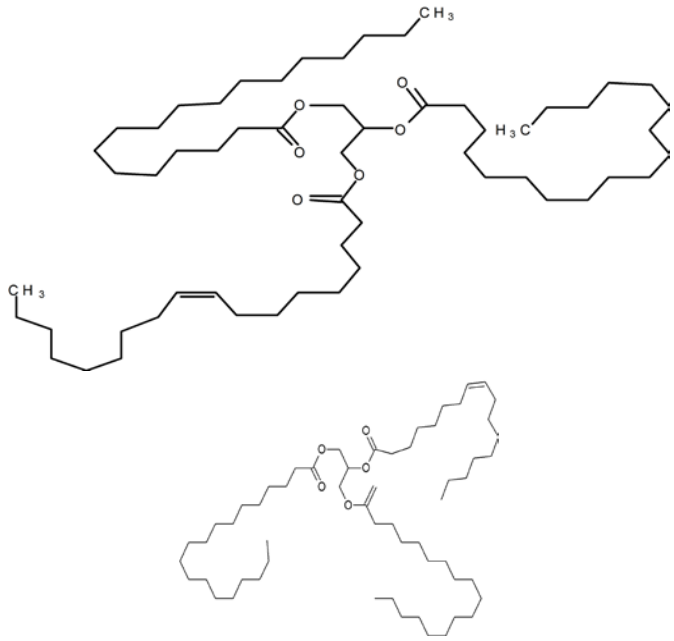

Figure 6. *Chemical structure of 1,3-distearyl-2-oleoyl glycerol*

The *Platonia* gender ("bacuri") is very rich in various natural substances as xanthones (euxantonas) **(A)**, fatty acids, and triglycerides. Studies with fruit pulp detected ascorbic acid **(B)**, and polyphenols as main bioactive compounds.[51] The phytochemical study of the hexane extract of the pericarp and methanolic the bacupari seeds, *Garcinia brasiliensis*, revealed the presence of prenylated benzophenones 7-epiclusianone **(C)** and guttiferone a **(D)**, respectively. In studies of volatile compounds of "bacuri" pulp analysis showed the presence of terpene alcohols, the most abundant linalool **(E)**. [52] The Garcinielliptone substance has isomers **(F1 and F2)**, is a benzophenone poliprenilada which was also isolated from the hexane extract of bacuri seeds, being a unique substance in *Platonia* gender.[48] The fruit of this species is rich in β-carotene, a compound of potent antioxidant activity.[53]

The species of the Annonaceae family are very consumed in the Northeast of Brazil, an example is the species popularly known as "ata" (*Annona squamosa* L.) and soursop (*Annona crasiflora* Mart.). *A. crassiflora* is a tree that produces a typical fruit known as the cerrado araticum, its fruits are eaten "in natura" by native people or used to make juice, ice cream or jelly. The oil from the seeds are used against leather infections hairy and in folk medicine the leaves and seeds for infusion are used against diarrhea and as antitumor. The *A. vepretorum* Mart. ("bruteira") species, is a native plant of the savanna of Brazil, its fruits are consumed as juice, being a species used in folk medicine in the treatment of inflammation, its leaves (decoction) are used in the bath in the treatment of skin, allergies and infections. [54]

Another genus of Annonaceae family with few known species distributed in neotropical regions (except Argentina and Paraguay), is the *Guatteria* (Ruiz & Pav.), the largest genus of Annonaceae. *Guatteria friesiana* (W. A. Rodrigues) is a tree known as "envireira" and "envira" found in Brazilian and Colombian Amazon Basin. The compounds isolated from species of the genera *Xylopia* and *Guateria* (*G.friesiana*, *G. blepharophylla* and *Xylopia leavigata*) and *Annona vepretorum* Mart. ("bruteira") were tested against the enzyme AChE showing promising results and good motivators for preclinical studies.[55]

They are reported in various literature actions of herbal extracts and/or isolated compounds of *Guatteria* and *Xylopia* genera (family Annonaceae) with cytotoxic, antifungal, antimicrobial, antioxidant and anti-parasitic activities and some of these activities may be relevant to the treatment of AD, justifying the selection of species *G. friesiana*, *G. blepharophylla* and *X. laevigata* used in this study for this possible therapeutic application. [50]

In figure 7 are reported various promising structures of the main compounds isolated from *P. insignis* ("bacuri") and family Clusiaceae.

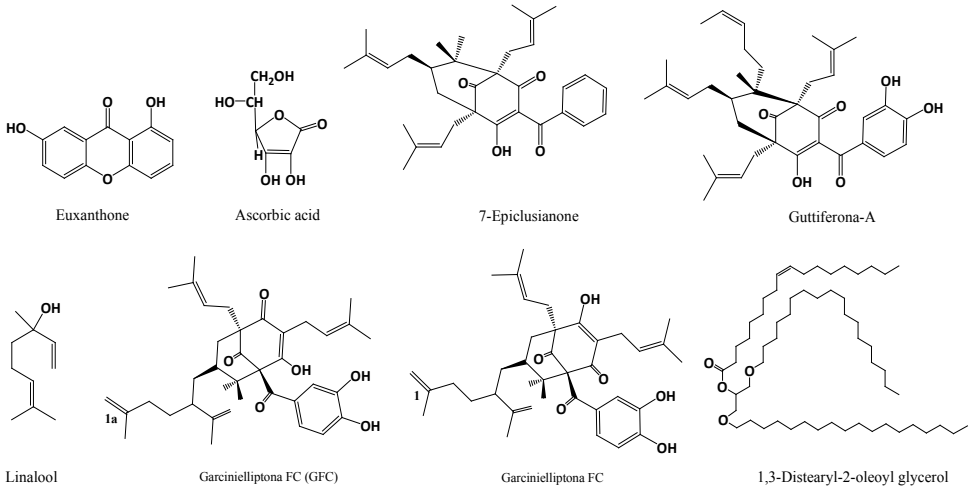

Figure 7. Structures of the main compounds isolated from *P. insignis* ("bacuri") and family Clusiaceae [50]

The compounds presented in Figure 8 were isolated from alkaloidal fraction of methanol extract of leaves and core of the species: *G. friesiana*, *G. blepharophylla*, *X. laevigata* and *A. vepretorumI.*[54,56] AChE inhibition tests were performed following the Ellman assay[33] modified by Rhee[22] the compounds namely: isomoschatoline, gualteriopsicine, demetoxiguadiscine, liriodenine, ent-3-β-hidroxicaurenóic acid, Kaurenoic and 6,6a-dihidrodimetoxiguadisine acid. The quantitative assay of *in vitro* AChE enzyme possible to calculate the values of the 50% inhibitory concentration for substances: isomoschatolina (IC_{50} = 49.00 μg.mL^{-1}), guatteriopsicina (IC_{50} = 0.3 μg.mL^{-1}), kaurenoic acid (IC_{50} = 375 μg.mL^{-1}); 6,6a-diidrodesmetoxiguadiscine (IC_{50} = 339 μg.Ml^{-1}), desmetoxiguadiscine (IC_{50} = 3021 μg.mL^{-1}), liriodenine (IC50 = 38 μg.mL-1) ent-3-β-hydroxy-acid caur -16-en-oic acid (IC_{50} = 248 μg.mL^{-1}) which showed significant results when compared to galanthamine that presents AChE inhibitory activity IC_{50} = 0,37x10^{-3} μg.mL^{-1} and rivastigmine IC_{50} = 1,87 μg.mL^{-1}. These values were obtained from the initial velocity of the samples with five different concentrations and compared with white speed, in this case the buffer solution.

The liriodenine (IC_{50} = 38 μg.mL^{-1}.), isomoschatoline (IC_{50} = 49 μg.mL^{-1}.) and guatteriopsicina (IC_{50} = 0.3 μg.mL^{-1}.) alkaloids exhibited better inhibition when compared to the positive standard (rivastigmine) IC_{50} = 1.87 ug. ml^{-1}).

Research with the substances isolated *X. laevigata*, *G. blepharophylla*, *G. friesiana* and *A.vepretorum* species (Figure 8) motivate conducting of *in vivo* tests to analyze its future application in pharmaceutical formulations for treatment of neurodegenerative diseases which depend on the modulation of the enzyme acetylcholinesterase, including AD.[55]

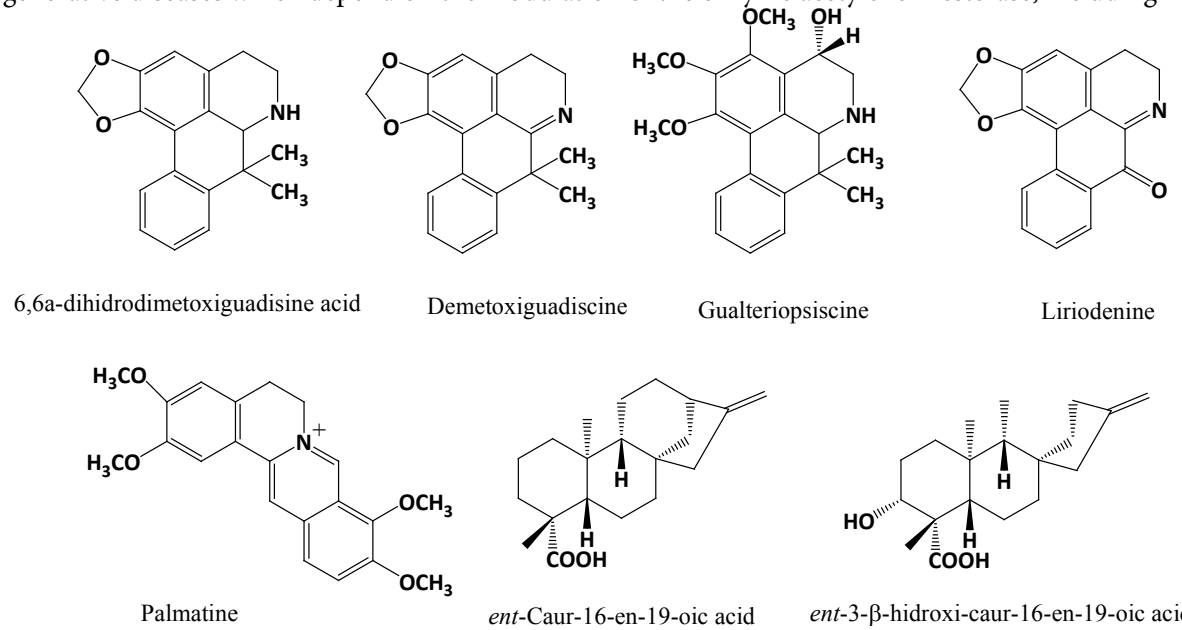

Figure 8. *Structure of the isolated constituent of the Guateria, Xylopia and Annona genera species*

Another motivator study was conducted through surveys of the species *Citrus limon* (L.) Burm (Rutaceae), a plant of northern and northeast of Brazil, popularly known as "Lemon Tree", whose leaves and fruits are taken advantage of by popular medicine for therapeutic purposes. In this perspective a new bioactivity study identified compounds with a potential inhibition of the enzyme acetylcholinesterase and carried out a qualitative and quantitative assessment of the activity of these compounds to verify their pharmacological properties with emphasis on prevention and/or treatment of AD. [57] The inhibitory effect of the mixture of constituents (5,8-dimethoxy- psoralen and 5,7-dimethoxycoumarin) isolated ethyl acetate extract (EtOAc) of leaves of *C. limon* (lemon) about acetylcholinesterase activity *in vitro* was evaluated by adjusting34 of the spectrophotometric method of Ellman.[10] In these *in vitro* studies with the species *C.*

limon (lemon) was observed an inhibition of AChE activity 95.9% when neostigmine was used at a concentration of 0.1 mg ml^{-1}. When evaluated the mixture of the isolated constituents of EtOAc extract of *C. limon* leaves at concentrations of 0.1; 0.05; 0.025; 0.0125 and 0.00625 $mg.mL^{-1}$ was detected inhibition 57.75; 49.89; 35.03; 23.78 and 8.71% in the AChE activity *in vitro*, respectively. Based on these results it was also determined the effective concentration 50% (EC_{50}) corresponding to 0.061 $mg.mL^{-1}$. In this perspective, the mixture of the individual constituents of the EtOAc extract of the leaves of *C. limon* (lemon), 5,8-dimethoxy-psoralen and 5,7-dimethoxycoumarin (Figure 09) showed a value lowerof EC_{50} than those measured values for extracts obtained from other species of medicinal plants that have been studied. Thus, the study suggests that the said fraction can demonstrate inhibitory results of the AChE activity *in vitro*, with potential application in experimental pharmacological models in rodents to assess their pharmacological potential in preclinical trials in the experimental procedures that simulate the pathologies related to central nervous system, in which the modulation of AChE responds by physiology inherent psychosocial disorders, particularly AD.

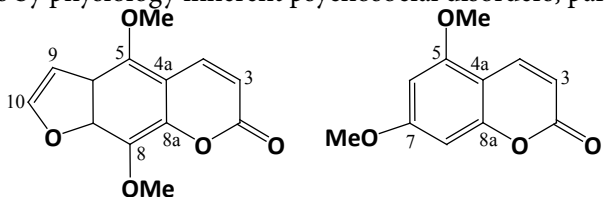

Figure 09. *Structure of AChE inhibitors in C. limon (lemon); A) 5,8-dimethoxy-psoralen and B) 5,7-dimethoxycoumarin*

Other fruits are reported in the literature as follows: pulp extracts, seeds and peels of fruits jenipapo (*G. americana* L.), "umbu" (*Spondia tuberosa* L.) and hog plum (*Spondia purpurea* L.) were evaluated in relation to antioxidant potential and anticholinesterase and showed very promising results. It was detected high antioxidant activity and acetylcholinesterase of chlorogenic acid, one of the major constituents present in the seeds of hog plum (*Spondia purpurea* L.). These findings, the acetylcholinesterase activity was carried out in comparison to physostigmine. The results suggested that these fruits are used as a source of antioxidant supplementation in human daily diet and these are also promising in the production of new medicaments.[58]

2.3 Natural products as candidates for drugs useful in the treatment of Alzheimer's disease

In recent decades, the Medicinal Chemistry has been seeking new alternatives and tools able to lead to greater agility, security and more efficient direction in the planning and prospecting of drug candidates.[59] In this perspectiva is clear that the plant kingdom has emerged as a major supplier of substances with various biological activities.[28] Of the new drugs that are approved many are derived from natural products. The search for new natural agents for the treatment of AD goes beyond acetylcholinesterase inhibitors, which aims to stabilize the levels of acetylcholine in the synaptic clefts to maintain neurotransmission, since the low acetylcholine levels in AD are a consequence of disease. Galantamine is an alkaloid isolated from various plant species of the family Amaryllidaceae. It is an inhibitor of acetylcholinesterase long-acting, selective, reversible, competitive, which produces beneficial effects even after the treatment.[30] Other alkaloids similar to galantamine also stand out by having a good inhibitory activity on AChE enzyme. The alkaloid, sanguinine present in *Eucharis grandiflora* (Amaryllidaceae), was ten times more active than the galantamine *in vitro* assays. Two other active derivatives of galantamine were isolated: 11-hidroxighalantamine, which showed similar activity to galantamine *in vitro* and epinorgalantamine[7] Three active alkaloids (type-lycorine) were isolated, the oxoassoanine the assoanine, pseudolicorine and the assoanina was more active with IC_{50} four times smaller than the galantamine control.[7] Huperzine a (huPA) a sesquiterpene alkaloid, isolated from *Huperzia serrata* (*Lycopodium serratum*) a plant of Lycopodiaceae family, is an alkaloid that acts as a powerful, highly specific and reversible inhibitor of AChE.

A triterpene, α-onocerine, isolated from the genus *Lycopodium* (Lycopodiaceae) also had a good acetylcholinesterase activity (IC_{50} = 5.2 $\mu mol.L^{-1}$) better than the donezepil.[1] The ginkgolides A and B, two diterpenes isolated from *Ginkgo biloba* (Ginkgoaceae), showed the ability to prevent neuronal death in response to damage caused by β-amyloid peptide. The study of 17 monoterpenes with skeleton p-menthane hydroperoxide, presents in several types of mint oil, about the inhibition of acetylcholinesterase showed that the ketone monoterpenes (-)-carvone, showed greater inhibitory activity than alcohols (+)-menthol. The (+)-pulegone was the most active monoterpene [34]

The fractionation of the methanol extract of *Angelica gigas* (Umbelliferae), led by inhibiting activity of AChE enzyme, led to the isolation of 12 coumarins. Five were active in inhibiting the enzyme acetylcholinesterase: decursinol, marmesine, xanthotoxine, isoimperatorine and nodakenine.[60]

Flavonoids, quercetin and myricetin, present in many vegetable and recognized antioxidant activity prevented the inactivation of muscarinic acetylcholine receptors by oxidative damage catalyzed by endogenous inhibitor of low molecular weight present 3 times more in the brains of Alzheimer's patients. The damage to these receptors causes a decrease in cholinergic transmission. The inactivation of the receptors by the inhibitor is a continuous decline factor for AD patients and the use of these flavonoids can increase the efficacy of acetylcholinesterase inhibitors.[61] The study with the phenolic constituents related to wine, myricetin, morin, quercetin, kaempferol, (+)-catechin and (-)-epicatechin allowed to evaluate its activities on training, extension and destabilization of β-amyloid fibrils. All polyphenols inhibited in a dose-dependent manner, the formation of Ab fibrils as well as its extension.[62] Another therapeutic approach for the treatment of AD is the inhibition of β-secretase (BACE1), the enzyme that cleaves APP (amyloid precursor protein)

generating Aß (β-amyloid peptide). In this model the constituents of green tea were tested, the (-)-gallocatechin gallate, the (-)-epigallocatechin gallate and (-)-epicatechin gallate, which showed a potent inhibitory activity. In addition to the inhibition of BACE1, the (-)-epigallocatechin gallate also has potent antioxidant properties and prevent neuronal damage induced by free radicals.[63,64] The flavonoids gossipina present in *Hibiscus vitifolius* (Malvaceae), baicalein and baicalina, isolated from *Scutellaria baicalensis* insulated (Labiatae) were evaluated in models of neurotoxicity induced by the Ab peptide and oxidative stress. In both tests, the compounds were shown to be active in protecting cortical cells.[65.66] In figure 10 shows some natural product inhibitors of AChE.

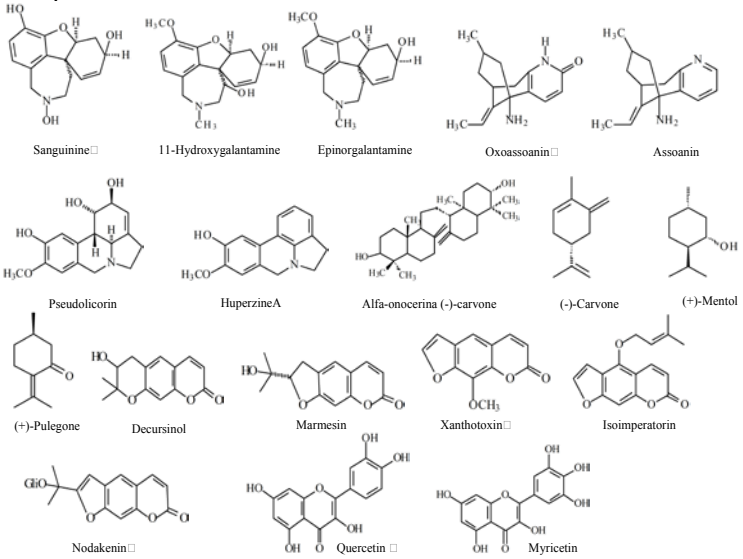

Figure 10. *Some substances obtained from natural product inhibitors of the enzyme AChE*

FINAL CONSIDERATIONS

In thi rewien we saw that in Brazil exist millions of patients with Alzheimer's disease and that this disease are associated with deficits in brain neurotransmitters, such as acetylcholine, noradrenaline and serotonin. The treatment is symptomatic and consists of the restoration of cholinergic function. In the process, raising the level of acetylcholine could be helpful to improve one of the signs of the disease, learning disabilities. Preclinical tests with animals and compounds acetylcholinesterase inhibitors isolated from the species *Citrus limon* (lemon), *Citrus sinensis* (orange), *Mangifera indica* (mango), *Kalanchoe brasiliensis* Camb. (white courama) *Platonia insignis* (bacurizeiro), among others, suggest a promising advance in the search for herbal from natural products. Some fruits that have antioxidant and inhibitory actions of AChE may be useful in the prevention or treatment of disease when used as dietary supplements. Therefore, it is of fundamental importance to disseminating information about the research that address the way that nutrition interferes in AD, either through the use of antioxidants, since this way you can prevent many people affected by DA become even more vulnerable, providing such a better quality of life, slowing the symptoms and progression of the disease.

ACKNOWLEDGMENT

The authors thank CNPq for scholarships

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Cite this article as:
Chistiane M. Feitosa, Antônio do Nascimento Cavalcante, Soane Kaline Morais Chaves , Lidiane S. Araújo . Medicinal plants of Brazil and Alzheimer's disease: Evolution in traditional use and pre-clinical studies. Asian Journal of Biomedical and Pharmaceutical Sciences, 6(58), 2016, 01-13.