Chapter 9

Citrus: A Perspective for Developing Phytomedicines for Neurodegenerative Diseases

Chistiane Mendes Feitosa,
Rivelilson Mendes de Freitas, Valéria Lima Silva,
Lidiane da Silva Araújo,
Cassio Herbert Santos de Melo and
Felipe Pereira Da Silva Santos

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66677

Abstract

The antioxidant and anti-inflammatory properties of *Citrus* flavonoids can play a key role in their activity against several degenerative diseases and particularly brain diseases. In Brazil promising studies deposited in the patents "pharmaceutical formulation" form was obtained from the inclusion of *Citrus sinensis* L. (orange) essential oil with β-cyclodextrin and *Citrus limon* (lemon) compounds and their applications in therapy of Alzheimer's disease. In this chapter, we report activities of active compounds present in the genus *Citrus*, which include antioxidant, anti-inflammatory, anxiolytic, insecticidal, and anti-cholinesterase activities. These activities are associated with some neurodegenerative diseases such as Alzheimer's disease (AD). Pharmaceutical formulations containing such compounds (for example, inhibitors such as acetylcholinesterase (AChE) of *C. limon* (5,8-dimethoxy-psoralen and 5,7-dimethoxycumarin) and essential oil from *C. sinensis* oils are reported in this chapter. These results indicate that the effects of the essential oil and substances of *Citrus* species are very interesting for further isolation of AChE inhibitors that can be used in the formulation of natural products for neurodegenerative diseases.

Keywords: citrus, *Citrus sinensis*, *Citrus limon*, acetylcholinesterase, neurodegenerative diseases

1. Introduction

Citrus fruits make up the largest sector of the global production of fruits, with more than 100 million tons produced every season [1]. *Citrus* is one of the most important genera of Rutaceae family because its fruits are estimated primarily as articles of diet [2]. The aromatic oils from

Citrus are used as flavoring agents in a variety of foods, drinks and confectionery and for fragrance applications. There are reports of the *Citrus* genus with important biological activities, namely antioxidant [3, 4], antimicrobial [5], anti-inflammatory [6], insecticidal [7, 8], anxiolytic [9], and anticholinesterase [3, 10]. In traditional Chinese medicine, the dried peel of *Citrus reticulata* (mandarin) has been widely used for centuries as a remedy for treating indigestion and fighting respiratory tract inflammatory syndromes such as asthma and bronchitis [1]. *C. aurantium* L., commonly known as sour orange bitter, is popularly used as a medicine in Brazil and other countries to treat anxiety and insomnia and used as an anticonvulsant, suggesting itcan act as a central nervous system (CNS) depressent [11]. *Citrus limon*, popularly known as lemon, was described possesesing variuos biological activities such as antifungal [12], antimicrobial [13], antioxidant [14, 15], antinoceptive [14], and larvicidal [16]. *C. limon* comprises flavonoids, volatile oils, and coumarins; however, their effect on the CNS is not well known [17].

In this chapter, we report activities of active compounds present in the genus Citrus, including antioxidant, anti-inflammatory, anxiolytic, insecticidal, and anticholinesterase activities, which are associated with some neurodegenerative diseases such as Alzheimer's disease and we report the pharmaceutical formulation of *C. limon* and *Citrus sinensis*.

2. Acetylcholinesterase activity (AChE) of Citrus species

Alzheimer's disease (AD) is a multifactorial disease that affects a significant portion of the population and its incidence has grown over the years due to the increasing proportion of elderly people in the world population. Factors such as formation of senile plaques and neurofibrillary tangles, reduction of acetylcholine levels (by inhibiting the enzyme acetylcholinesterase) and oxidative phenomena are related to the development and/or progression of AD [18, 19]. The acetylcholinesterase (AChE), an enzyme inhibitor associated with AD, is widely detected by Ellman's test. According to the principle of the method of Ellman et al. [18], the reaction with the thiol has been shown to be sufficiently rapid so as not to be rate limiting in the measurement of the enzyme and in the concentrations used does not inhibit the enzymatic hydrolysis [18, 19].

Some AChE inhibitors are found naturally in medicinal plants. Reversible cholinesterase inhibitors are currently used in clinical trials for treatment of Alzheimer's disease [4]. The treatment is based on the inhibition of AChE, which hydrolyzes acetylcholine, increasing their availability to cholinergic transmission [20].

The anticholinesterase activity of extracts from *C. limon* (lemon) leaves compared to galantamine, a drug widely used in the treatment of AD, and other species used in popular medicine in Northeast Brazil are reported in a phytochemical screening [21]. Another study described inhibition of a fraction of the ethyl acetate extract from the leaves of *C. limon,* which was isolated from the active fraction named two coumarins: 5,8-dimethoxy-psoralen and 5,7-dimetoxi-coumarin (**Figure 1**). *In vitro* studies indicate IC_{50} of 5.8 μg mL^{-1} (95% confidence) and *in vivo* studies with male Swiss mice showed inhibition of 30.09–30.06% for the enzyme AChE compared to neostigmine, a drug used in the treatment of AD [11].

http://dx.doi.org/10.5772/66677

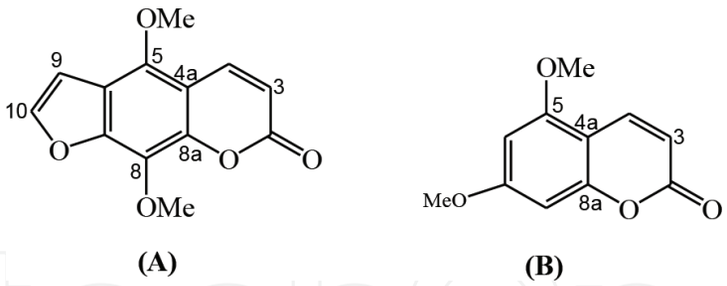

Figure 1. Inhibitors AChE of *C. limon* (A) 5,8-dimethoxy-psoralen (B) 5,7-dimethoxycumarin. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

In vitro and *in vivo* assays with the essential oil of *C. sinensis* (L.) Osbeck (orange) against AChE enzyme indicated that there was a significant decrease in AChE activity in the hippocampal region of mice in *in vivo* tests. *In vitro* testing of *C. sinensis* essential oil showed a value inhibition concentration with IC_{50} = 63 μg mL^{-1} whereas for the standard (neostigmine) was obtained as IC_{50} value = 1.87 μg mL^{-1}. For the antioxidant, activity was a significant 20% reduction in the hippocampus of mice treated with 150 mg kg^{-1}on lipid peroxidation, thereby reducing oxidative stress and nitrite content, these doses showed a significant reduction in all groups, suggesting a neuroprotective effect against brain injury [4].

Studies on the species as *Citrus medica* L. cv. Diamante (cidra) demonstrated anticholinesterase activity, which can be explained by the high amount of monoterpenes present in the skin of the fruit [10]. According to studies 17 monoterpenoids with *p*-methane skeleton was reported, the AChE inhibitory activity of compounds such as γ-terpinene and terpinen-4-ol arrive at 22.6 and 21.4% at 1.2 mM, respectively. Other terpenes such as limonene and linalool present in 164 μg mL^{-1} concentration, inhibition of the 27 and 37%, respectively. The same activity is presented to study the species such as *Citrus hystrix* (Combava), which caused 10% inhibition of AChE enzyme and that this action was related to the presence of acyclic and monocyclic monoterpenes such as citronellal and β-phellandrene, respectively, present in the essential oil extracted from the leaves of this plant [22].

The structural diversity of terpenoids that exert inhibitory activity of AChE is difficult to predict the potential structure-activity relationship. But it is known that some features, such as the presence of a hydrophobic ligand, may be associated with greater effectiveness in the inhibition, since the active site of AChE is known to be susceptible to hydrophobic interactions. The monoterpenes consist of a hydrocarbon skeleton that can be cyclic (*α*-pinene) or acyclic (linalool), a feature that may also influence their AChE inhibitory activity. For a bicyclic monoterpene skeleton pinene or carene, the potential of AChE inhibition was associated with the position of the double bond [10]. The presence of terminal olefins ($H_2C{=}CH_2$) resulted in decreased inhibition of AChE, as well as the presence of an oxygenated functional group [20].

Experiments that assess memory evaluate the effects of acute treatment with the essential oil of leaves (EOL) from *C. sinensis* in the acquisition of spatial memory in rats using the paradigm of the Morris water maze. In Morris assay, the acquisition of memory space is evaluated by time the animal takes to locate the platform after having been trained [23].

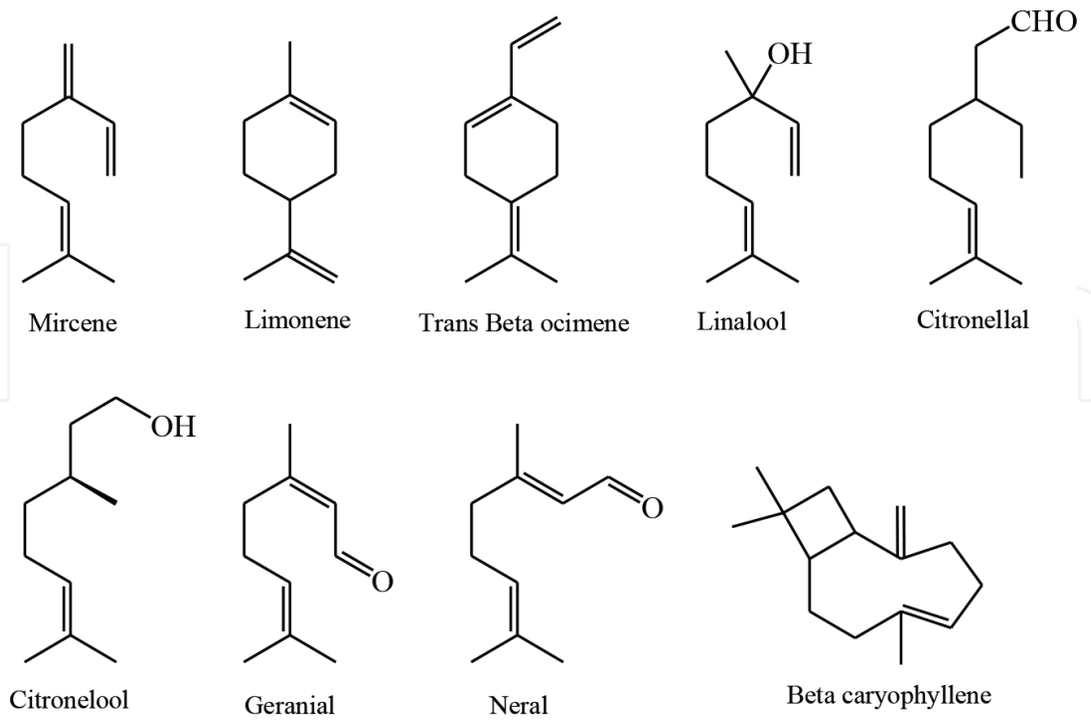

Figure 2. Constituent chemicals of the *C. sinensis* essential oil. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

The composition of EOL of *C. sinensis* is mainly composed of the class of monoterpenes such as limonene (24.14%), citronellol (30.42%), and geranial (31.42%) (**Figure 2**). The animals were previously untreated with doses of EOL of *C. sinensis* 50, 100, and 200 mg kg^{-1} and the open field test conducted and the Morris water maze task [23].

The acquisition of memory space is evaluated by time the animal takes to locate the platform after having been trained. The results of the open field were demonstrated that animals do not exhibit motor stimulus when treated with the essential oil of *C. sinensis* and the results of water maze were significantly lower to find the submerged platform than the negative control group ($p < 0.01$) indicating an increased memory capacity in the treated animals, but must be reinforced by other memory tests recommended by the literature. These results indicated that the effects of the EOL of *C. sinensis* may involve the cholinergic system and produce a reversal of memory impairment, caused by over activity of AChE [4].

Activities of active compounds present in the genus Citrus, which include activities such as antioxidant, anti-inflammatory, anxiolytic, insecticidal, and anticholinesterase. These activities are associated with some neurodegenerative diseases such as Alzheimer's disease [24].

3. Citrus antioxidants

Oxidative stress produced by free radicals has been implicated in the pathogenesis and progression of a wide variety of clinical disorders such as cancer, cardiovascular disease, inflammation, epilepsy, diabetes, and Alzheimer's disease [25]. Oxidative stress is the result of natural deficiency of antioxidant defenses, or by increased levels of reactive species derived

http://dx.doi.org/10.5772/66677

from oxygen [26]. Reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•) are produced as a result of many biochemical reactions and can be considered the main cause of oxidative damage, as protein denaturation and lipid peroxidation mutagenesis [10, 27, 28].

The plants of the Citrus genus are rich in compounds that have antioxidant properties [22]. Phenolic compounds, particularly flavonoids, have shown an important antioxidant activity, which is mainly based on their structural characteristics and other chemical characteristics due to the number and position of phenolic hydroxyl groups [29].

Natural antioxidants from fruit juices offer an alternative source of dietary ingredients to promote healthy life. A recent study on the juice of *C. sinensis* (L.) Osbeck suggested the influence of C-and O-glycosyl flavonoid antioxidants and anticholinesterase properties [24, 30]. From the juice, 12 individual components were identified for the first time, namely, four C-glycosyl flavones (lucenin-2, vicenin-2, stellarin-2, lucenin-2 40-methyl ether, and scoparin), three flavonol derivatives (quercetin-3-O-(2-rhamnosyl)-rutinoside, quercetin-3-O-hexoside, quercetin 3-hydroxy-3-methylglutaryl-glycoside), O-triglycosylflavanone (narirutin 4-O-glucoside), and a flavones O-glycosides (chrysoeriol 7-O-neoesperidoside). Moreover, the influence of the identified C- and O-glycosyl flavonoids on the antioxidant and acetylcholinesterase activity of these juices has been evaluated [30].

Thirty-four types of essential oils of Citrus and its components were investigated for their antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH). The activity of components compared with a standard antioxidant, the trolox showed effects on DPPH scavengers ranging between 18 and 64%. One possible explanation for the difference in efficiency found in this study may be substantial variation in the compounds of the essential oils of citrus. Among the 34 types of essential oils, the radical scavenging activity of the lemon (*C. ichangensis*) species was the largest compared to oils Tahiti limon (*C. aurantifolia*) and eureka lemon (*C. limon*). Higher antioxidant activity of the high amount of terpenes with the exception of limonene and myrcene are reported [5]. Generally, the high radical scavenging activity was found when large quantities of terpenes include γ-terpinene and terpinolene. In Mexican lemons, Tahiti, eureka, and lisbon, the combined percentage of neral and geranial varied from 1.7 to 3.5%. It was also found that the components neral and geranial contribute to its effect sequestering these samples. *C. sinensis* and *C. reticulata* most effective compounds such as γ-terpinene and terpinolene were present in small amounts [5]

The antioxidant effect of α-terpinene, nootkatone, citronellal, citral, γ-terpinene, terpinolene, and geraniol substances result showed greater than trolox ($p < 0.05$). According to calculations based on the height of the peak of DPPH, γ-terpinene (84.7%), terpinolene (87.4%), and geraniol (87.7%) had radical scavenger effect 3.5 times that of trolox. Significant differences ($p < 0.05$) were not found between decanal and geranyl acetate. The antioxidant activity of linalool, citronellol, α-pinene, and octanal (18.7–22.4%) was higher than that of α-terpineol, octanol, myrcene, decanol, β-pinene, terpen-4-ol, *p*–cymene, and *d*-limonene (8.8 to 16.5%) [3]. Subsequent studies demonstrated a significant *in vitro* antioxidant activity of α-terpineol against lipid peroxidation inhibition of nitrite ions and hydroxyl radical [5].

The low-density lipoprotein plasma (LDL) plays a significant role in the development of atherosclerosis. The antioxidant activity of the essential oil of three Citrus species (*C. natsudaidai*,

C. hassaku, and *C. unshiu*) was evaluated and showed promising effects. Studies have shown the antioxidative effect to evaluate the effect of citrus essential oil components on human LDL *in vitro*. Among the authentic volatile compounds, tested gamma-terpinene showed the strongest antioxidative effect, and inhibited both the Cu^{2+}-induced and AAPH-induced oxidation of LDL. Gamma-terpinene added after 30 min (mid-lag phase) and 60 min (propagation phase) of incubation of LDL with Cu^{2+} inhibited LDL oxidation [31].

4. Citrus anti-inflammatory activity

The inflammation is typically characterized by an increase in tissue permeability and endothelial leukocyte influx of blood into the interstitium, causing edema. Different mediators influence each step of the cascade of inflammation and characteristically inflammatory agents exhibit therapeutic properties by blocking the action or synthesis of these mediators. While inflammation is a normal response to tissue injury, often it is uncontrolled in chronic autoimmune diseases such as rheumatoid arthritis and Crohn's disease, or when related to allergic response such as asthma and anaphylactic shock. In these cases, anti-inflammatory compounds are administered therapeutically to control the inflammatory response [32].

Plants rich in certain flavonoids have traditionally been used for its anti-inflammatory properties, being increasingly reported the isolation of flavonoids, including the Citrus genus with anti-inflammatory potential [6]. Citrus flavonoids appear to impact blood and microvascular endothelial cells [33].

Studies reported that nobiletin (flavone) is a major component in juice from *Citrus depressa*, it inhibits the invasive activity of human fibrosarcoma HT-1080 cells not only by suppressing the expression of matrix metalloproteinases but also by augmenting metalloproteinases-1 production [34, 35]. Also, nobiletin prevents tumor-cell invasion due to a decrease of metalloproteinase-9 production in the peritoneal dissemination of human gastric carcinoma in severe combined immunodeficient mice [36].

Therefore, these results further support the notion that nobiletin is likely to be a candidate for characterization as a novel immunomodulatory and anti-inflammatory drug [34].

Citrus peels, the dominant residue, possess a large variety of bioactive compounds; they are considered as potential sources of functional components [37]. In traditional herbal medicine in Korea, the dried fruit peels of *Citrus reticulata* have been widely used for centuries as a remedy to treat indigestion and to improve inflammatory syndromes of the respiratory tract such as bronchitis and asthma [38].

5. Citrus insecticidal

The insecticidal properties have been recognized in the essential oil of many species of the Citrus genus and various products containing (+)-limonene, linalool, and crude extract of some species of *Citrus* are already been sold [39].

http://dx.doi.org/10.5772/66677

Analyses were performed with the potential insecticidal activity by spraying volatile extracts from the bark of species two of orange–*C. sinensis* and *C. aurantifolia* [7]. Both exhibited, to varying degrees, insecticidal activity against mosquito, fly (*Musca domestica* L.) and cockroach. Insecticidal activity was better in the longer period of exposure (60 minutes) compared to 30 minutes of spraying. The volatile extracts of *C. sinensis* showed best potential insecticide and cheap. *Blattella germanica* L) was the species most susceptible to the effects of orange peel among the three insects studied. Later studies examined the ability of the insecticide essential oil *C. sinensis* in flies [7].

The observed effect of oil extracted from *C. limon* on the larvae *Culex pipiens* (house mosquito) and found a positive relationship between the exposure time and the percentage of mortality of larvae [8]. The extract from the bark of *C. aurantium* (L.) was evaluated for its toxicity against fly of olive (*Bactrocera oleae*), and fly of mediterranean (*Ceratitis capitata*). The flies of the species *B. olea*e to extract were more likely than *C. capitata* in residual contact bioassays. Both sexes of *B. oleae* were equally susceptible in both tests. However, males of the *C. capitata* were more likely than females, which can be best explained by their ability to metabolize chemical insecticides [39].

The herbal extracts to inhibit AChE activities are promising for the symptomatic treatment of Alzheimer's disease, a neurodegenerative disease that initially affects memory and thinking ability. Research indicates that in the literature, there is a growing search for new inhibitors of AChE activity in plant extracts [40]. This search is directed mainly to plants already used in traditional medicine for the treatment of insomnia, amnesia, depression, and anxiety, or to extend longevity and improve memory and cognitive function. Some plants, such as *Centella asiatic*a L. (centelha-asiática) (Umbeliferae) and *Ginko biloba* L. (ginkgo) (Coniferae), used in traditional Indian and Chinese medicine, demonstrated in studies of pharmacological activities, relevant results in the treatment of cognitive disorders, anticholinesterase, anti-inflammatory, and antioxidant actions. In light of these results, these plants have been indicated for therapeutic use in the treatment of Alzheimer's disease [24].

The inhibitory activity of AChE is a significant effect induced by coumarins and particularly monoterpenes such as *d*-limonene. It is also due to the presence of *d*-limonene from Citrus extracts that showed toxic actions in insects, representing a potential alternative to chemical insecticides and pharmacological promising to search for inhibitors of the enzyme AChE [41].

6. Terpenes acetylcholinesterase inhibitors as perspective for the production of herbal medicines

Pharmacological treatments most commonly employed for Alzheimer's disease (AD) stands out the use of materials whose action increases central cholinergic function, increasing levels of acetylcholine in the brain through inhibition of the enzyme acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). These enzymes are responsible for the hydrolysis of acetylcholine to acetate and choline, which prevents its action to the neurotransmitter. The inhibition of the enzyme AChE has been investigated for the treatment of various neurological

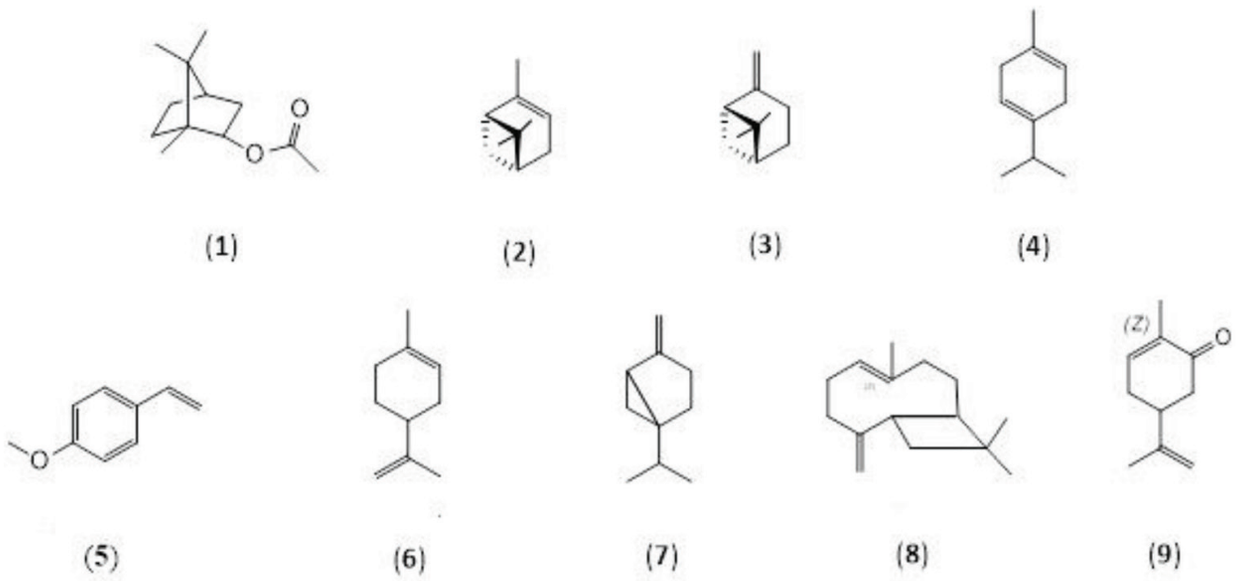

Figure 3. Terpenes AChE and/or BuChE inhibitors (ethyl bornila (1) α-pinene (2), β-pinene (3), γ-terpinene (4), trans-anethole (5), (+) - limonene (6) and (+) - sabinene (7) α-caryophyllene (8) and (-)- - carvone (9). Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

diseases, and are therefore as the most suitable strategy for the treatment of AD and other diseases such as: senile dementia, ataxia, myasthenia gravis, and Parkinson's disease [42].

Various terpenes present in plant essential oils are reported in the literature showing inhibitory activity against AChE and BuChE for example: ethyl bornila (1) α-pinene (2), β-pinene (3) γ-terpinene (4), *trans*-anethole (5), (+)-limonene (6), (+)-sabinene (7), α-caryophyllene (8), and (-)-carvone (9) [24] (**Figure 3**). In promising studies it was shown that *trans*-anethole (5) compound exhibited the strongest activity against AChE and BuChE, with IC_{50} values of 134.7 and 209.6 ng mL^{-1}, respectively [43].

Promising studies show that terpenes can have their activity potentiated by complex with cyclodextrins making it a promising source of possible pharmaceutical formulations. The inclusion complex obtained between β-cyclodextrin and *p*-cymene terpene potentiated analgesic and anti-inflammatory activities of this monoterpene [44]. The thermal instability, poor solubility in water, and the highly volatile compounds are some of the properties of essential oils or terpenes that by complexing with cyclodextrins improves its technological applicability [45, 46].

7. Pharmaceutical formulation of citrus

The cyclodextrins (CDs), for example β-cyclodextrins (**Figure 4**), are composed of complex carbohydrates glucose units (α-D-glucopyranose) joined by α-1,4 linkages type, with a structure similar to a trunk cone. In 1903 Franz Schardinger identified cyclodextrins, as products resulting from the degradation by the action of amylase enzyme. Cyclodextrin starch glycosyl is a compounds of CDs that have played an important role in Medicinal Chemistry with regard to controlled release technology of drugs, currently represents one of the boundaries of science

http://dx.doi.org/10.5772/66677

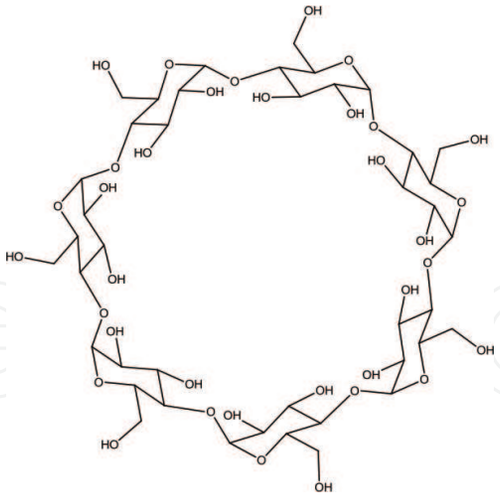

Figure 4. Structure of β-cyclodextrin. Seven units of D – (+)-glucopyranose. The CDs have a hydrophobic central cavity and a hydrophilic surface. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

involving different aspects multidisciplinary knowledge. The delivery systems, often described as a "drug delivery systems," offer numerous advantages when compared to other conventional dosage [47].

In the pharmaceutical industry, the CDs are used mainly to improve stability and bioavailability of the active principle and its organoleptic properties such as taste and smell in pharmaceutical formulations. The CDs can also be used to mask the unpleasant smell and taste of certain drugs, transforming solid liquid compounds, reduce volatility, and avoid undesirable mismatches. The increased solubility, dissolution, modification of the pharmacokinetics, and controlled release of drugs are other CD applications. The CDs have been used successfully in the management and drug delivery by various routes and/or administration sites such as oral, vaginal, rectal, nasal, ophthalmic, pulmonary, dermal, and transdermal [47]. **Figure 4** shows the structure of β-cyclodextrin is widely used as an excipient in pharmaceutical industry at low cost and the size of the cavity, which is suitable for encapsulating most substances

In Brazil a pharmaceutical formulation from the essential oil inclusion complex of *C. sinensis* was obtained with β-cyclodextrin and their applications in therapy of disease Alzheimer's (AD). The aim of the present study was to explore the essential oil from leaves of *C. sinensis* (EOLCS) and inclusion complex with the cyclodextrin complexing and their use as a candidate drug in the formulation pharmaceuticals for the prevention and/or treatment of AD. These properties were determined from experimental models where biological application of essential oil (EOLCS) and inclusion complex with cyclodextrin complexing. Inclusion complex with (EOLCS) was proved experimentally by evaluating the inhibitory effect on the activity of the enzyme AChE *in vitro* and *in vivo* (deposit patent number BR 10 2016 0018552).

For the characterization inclusion complex of cyclodextrin and EOLCS was held the infrared spectroscopy and differential scanning calorimetry with three proportions, β-CD, and EOLCS, denominate of misture physical. It proved the formation of EOLCS complex with β-cyclodextrin through the analysis of differential scanning calorimetry, infrared spectra disappearance of the

groups, and thermogram and it can be concluded that the ratio is 6:94 IC greater stability. It was shown in preliminary studies that the EOLCS significantly inhibits acetylcholinesterase a total of 73% in group 50 mg kg^{-1}, 83% in group 100 mg kg^{-1}, and 76% 200 mg kg^{-1} and significantly improves memory of the animals in promising preclinical studies.

The *Citrus sinensis* results show a significant pharmacological effect of the compound isolated in the inhibition of AChE enzyme activity, and this action of great interest in the development of new phytomedicine for the prevention and/or treatment of AD. The results of this effort were so promising that we decided to deposit (BR 10 2016 0018552 deposit number) a request for a patent, aiming to protect the innovations developed.

The composition of the active fraction in *C. limon* (L.) Burm leaves is composed of a mixture of two coumarins, 5,8-dimethoxy-psoralen and 5,7-dimethoxycoumarin (**Figure 1**), identified by ^{1}H and ^{13}C NMR data analysis, and others experiments. It was also demonstrated that this mixture presents qualitative and quantitative AChE inhibition. *In vitro* studies indicated a CE_{50} value of 340 μg/mL with 95% of confidence. *In vivo* studies (10 and 25 mg/kg) revealed inhibition of 30.09 and 30.06% of AChE activity in relation to neostigmine, respectively. The deposited formulations suggest that the isolated fraction *C. limon* (L.) containing (5,8-dimethoxy-psoralen and 5,7-dimethoxycoumarin) can demonstrate inhibitory results of the AChE activity *in vitro* and *in vitro*, with potential applications in neurodegenerative diseases dependent on the modulation of this enzyme, including the Alzheimer's disease [28]. The sample containing the mixture of isolated components showed no toxic properties in rodents, since none of the animals treated with the doses of 10 and 25 mg/kg died during the 24-hour observation period. Suggesting that the said compound can be used in lower dose and may increase more effectively cholinergic stimulation.

Citrus species are very interesting for further isolation of AChE inhibitors which can be used for neurodegenerative diseases for example Alzheimer's disease application (**Figure 5**).

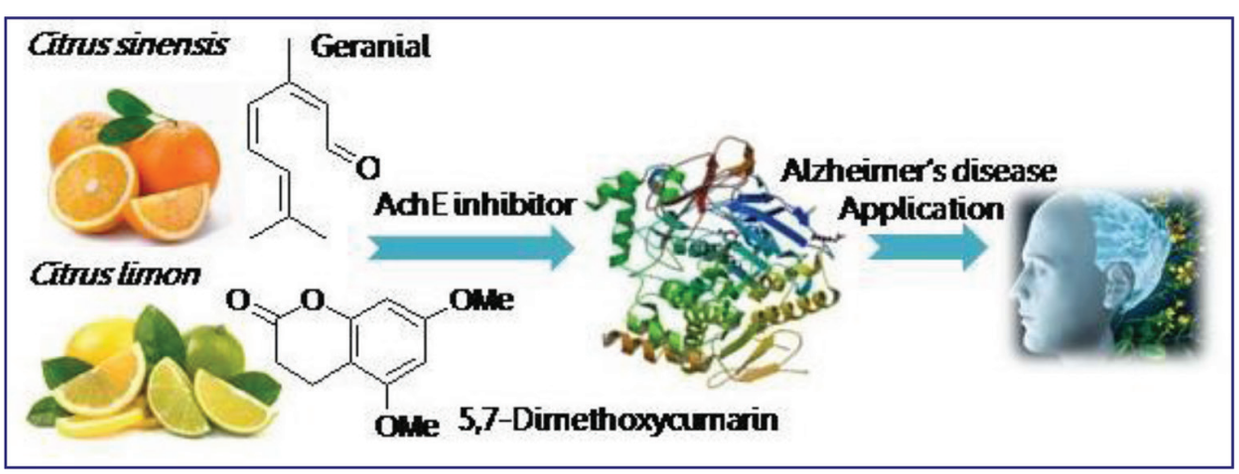

Figure 5. Pharmaceutical formulations were obtained from compounds and essential oils of *C. limon* (e.g., 5,7-dimethoxycoumarin) and *C. sinensis* (e.g., geranial). These compounds and essential oils were evaluated in preclinical trials on mice by memory tests and enzyme acetylcholinesterase inhibitors and proved to be very promising for potential applications in the treatment of neurodegenerative diseases such as Alzheimer's disease. Molecular structures were drawn with ChemDraw13 (Perkin Elmer).

http://dx.doi.org/10.5772/66677

Promising preclinical studies were conducted with compounds isolated from some of these Brazilian species others namely *Platonia insignis* (bacurizeiro), *Mangifera indica* (mango), and *Kalanchoe brasiliensis* (courama branca), for the prevention or progression of Alzheimer's disease [48].

8. Concluding remarks

The results confirm that AChE inhibitors as alternatives for preparation of phytomedicines are used in therapeutic treatment of AD, being plants the principal source of these inhibitors. Recent studies show that the terpenes may have intensified activities through inclusion complexes with cyclodextrins excipient pharmaceutical, making them a promising source for potential pharmaceutical formulations.

In general, the formulations reported in this chapter from essential oils or their constituents is in progress, as they contribute to various activities of plants and open perspectives regarding the chemical composition of bioactive metabolites of the studied species and are considered promising alternatives to discoveries of new chemical compounds of pharmaceutical interest.

Author details

Chistiane Mendes Feitosa*, Rivelilson Mendes de Freitas (in memorian)[#], Valéria Lima Silva, Lidiane da Silva Araújo, Cassio Herbert Santos de Melo and Felipe Pereira Da Silva Santos

*Address all correspondence to: chistiane@ufpi.edu.br

Federal University of Piaui, Campus Minister Petrônio Portela, Ininga, Teresina, Piauí, Brazil

[#]The author died recently after contributing with the research

References

[1] Ho SC; Lin CC. Investigation of heat treating conditions for enhancing the anti-inflammatory activity of citrus fruit (*Citrus reticulate*) peels. Agric Food Chem 2008, 56(17): 7976–82.

[2] Waheed A; Mahmud S; Saleem M; Ahmad T. Fatty acid composition of neutral lipid: classes of Citrus seed oil. J Saud Chem Soc 2009,13: 269–272.

[3] Campêlo LML; Almeida AAC; Freitas RLM; Cerqueira GS; Sousa GF; Saldanha GB; Feitosa CM; Freitas RM. Antioxidant and antinociceptive effects of *Citrus limon* essential oil in mice. J Biomed Biotechnol 2011, 2011: 678–673.

[4] Sá CG; Cardoso KMF; Freitas RM; Feitosa CM. Effect of oral treatment with essential oil of *Citrus sinensis* (L) Osbeck in the retention of spatial memory in rats evaluated in the Morris water maze. Rev. Ciênc Farm Basic Appl 2012: 33, 21–215.

[5] Choi HS; Song HS; Ukeda H; Sawamura M. Radical scavenging activities of Citrus essential oils and their components: detection using 1,1-Diphenyl-2-picrylhydrazyl. J Agr Food Chem. 2000, 48: 9, 4156–4161.

[6] Benavente-Garcia O; Castillo J. Update on uses and properties of citrus flavonoids: new findings in anticâncer, cardiovascular, and anti-inflammatory activity. J Agr Food Chem 2008, 56, 6185–6205.

[7] Rossi YE; Palacios SM. Fumigant toxicity *Citrus sinensis* essential oil in *Musca domestica* L. adults in absence and presence of a P450 inhibitor. Acta Tropica 2013, 127, 33–37.

[8] Siskos EP; Konstantopoulou MA; Mazomenos BE. Insecticidal activity of Citrus aurantium peel extract against Bactrocera olea and Ceratitis capitata adults (Diptera: Tephritidae). J Appl Entomol 2009, 133: 108–116.

[9] Almeida AAC; Costa JP; Carvalho RBF; de Sousa DP; Freitas RM. Evaluation of acute toxicity of a natural compound (+)-limonene epoxide and its anxiolytic like action. Brain Res 2012, 1448: 56–62.

[10] Conforti F.; Statti GA; Tundis R; Loizzo MR.; Menichini F. In vitro activities of *Citrus medica* L. cv. Diamante (Diamante citron) relevant to treatment of diabetes and Alzheimer's disease. Phytother Res. 2007, 21: 427–433.

[11] Carvalho RBF; Freitas RM; Lima LS.; Davi da JP; Feitosa CM. Chemical composition and anticholinesterase activity of an active fraction of extract of *Citrus limo*n (L.) Burm leaves. Quim Nova 2013, 36: 1375–1379.

[12] Ezzat SM. In vitro inhibition of Candida albicans growth by plant extracts and essential oils. World J. Microbiol Biotechnol 2001, 17: 757–759.

[13] Gutkind GO; Martino V; Granã N; Coussio JD; Torres RA. Screening of South American plants for biological activities. 1. Antibacterial and antifungal activity. Fitoterapia 1981, 1: 213–218.

[14] Campêlo LML; Gonçalves FCM; Feitosa CM; Freitas RM. Antioxidant activity of *Citrus limon* essential oil in mouse hippocampus. Pharmac Biol 2011, 49: 709–715.

[15] Luzia DMM; Jorge N. Antioxidant activity of lemon seed extract (Citrus limon) added to soybean oil in accelerated incubator-storage test. Quim Nova 2009, 32: 946–949.

[16] Furtado RF; de Lima MGA; Andrade Neto M; Bezerra JNS; Silva MGV. Larvicidal activity of essential oils against *Aedes aegypti* L. (Diptera Culicidae). Neotrop Entomol 2005, 34: 843–847.

[17] Vendruscolo GS; Rates SMK; Mentz LA. Chemical and pharmacologic data on medicinal plants used by the community of the Ponta Grossa neighborhood, Porto Alegre, Rio Grande do Sul, Brazil. Braz J Pharmacog 2005, 15: 361–372.

[18] Ellman GL; Courtney D; Andres V; Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961, 7: 88–95.

[19] Rhee IK; Van de Meent M; Ingkaninan K; Verpoorte R. Screening for acetylcolinesterase inhibitors from Amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. J. Chromatography A 2001, 915: 217–223.

[20] Miyazawa M; Yamafuji C. Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. J Agric Food Chem 2005, 53: 1765–1768.

[21] Feitosa CM; Freitas RM.; Luz NN; Bezerra MZ; Trevisan MT. Acetylcholinesterase inhibition by somes promising Brazilian medicinal plants. Braz J Biol 2011, 71: 783–789.

[22] Chaiyana W; Saeio K; Hennink WE; Okonogiet S. Characterization of potent anticholinesterase plant oil based microemulsion. Int J Pharmac 2010, 401: 32–40.

[23] Morris RGM; Garrud P; Rawlins JNP; O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982;297: 681–683.

[24] Howes MJR; Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav 2003, 75: 513–527.

[25] Wiegman CH; Michaeloudes C; Haji G; et al. Oxidative stress–induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2015, 136, (3): 769–780.

[26] Silva AO; Oliveira FRAM; Lima TC; Sousa DP; Souza AA; Freitas RM. Evaluation of the antioxidante effects in vitro of the isopulegone. Free Rad Antiox 2012, 2: 50–55.

[27] Tomé AR; Ferreira PMP; Freitas RM. Inhibitory action of antioxidants (ascorbic acido r alfa-tocopherol) on seizures and bran damage induced by pilocarpine in rats. Arq Neuro-Psiq 2010, 68: 355–361.

[28] Feitosa CM; Nunes AAC; David JM; David JPL; Lima LSL; Freitas RM; Carvalho RBF. Patent deposit number (BR 102012 008099-0 A2), 2013.

[29] Anagnostopoulou MA; Kefalas P; Papageorgiou VP; Assimopoulou AN; Boskou D. Radical scavenging activity of various extracts and fractions of sweet Orange flavedo (*Citrus sinensis*). Food Chem 2006, 94: 19–25.

[30] Barreca D; Gattuso G; Laganà G; Leuzzi U; Bellocco E. C- and O-glycosylflavonoids in Sanguinello and Tarocco blood orange [*Citrus sinensis* (L.) Osbeck] juice: identification and influence on antioxidant properties and aceylcholinesterase activity. Food Chem 2016, 196: 619–627.

[31] Takahashi Y; Inaba N; Kuwahara S; Kuki W. Antioxidative effect of citrus essential oil components on human low-density lipoprotein in vitro. Biosci Biotechnol Biochem 2003, 67: 195–197.

[32] Robbins S; Kumar V; Abbas AK. Pathological Basis of Disease, 7th ed.; Elsevier Saunders, Philadelphia, 2005.

[33] Manthey JA; Grohmann K; Guthrie N. Biological properties of Citrus flavonoids pertaining to cancer and inflammation. Curr Med Chem 2001, 8: 135–153.

[34] Lin N; Sato T; Takayama Y; Mimaki Y; Sashida Y; Yano M; Ito A. Novel anti-inflammatory actions of nobilein, a citrus polymethoxy flavonoid, on human synovial fibroblastos and mouse macrophages. Biochem Pharmacol 2003, 65: 2065–2071.

[35] Stetler-Stevenson WG; Aznavoorian S; Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. Annu Rev Cell Biol 1993, 9: 541–573.

[36] Sato T Koike L; Miyata Y; Hirata M; Mimaki Y; Sashida Y; Yano M; Ito A. Inhibition of activator protein-1 binding activity and phosphatidylinositol 3-kinase pathway by nobiletin, a polymethoxy flavonoid, results in augmentation of tissue inhibitor of metalloproteinases-1 production and suppression of production of matrix metalloproteinases-1 and 9 in human fibrosarcoma HT-1080 cells. Cancer Res 2002, 62: 1025–1029.

[37] Schieber A; Stintzing FC; Carle R. By-products of plant food processing as a source of functional compounds: recent developments. Food Sci Technol 2001,12: 401–413.

[38] Jung K H; Ha E; Kim MJ; Won H J; Zheng LT; Kim H K; Hong SJ; Chung JH; Yim SV. Suppressive effects of nitric oxide (NO) production and inducible nitric oxide synthase (Inos) expression by *Citrus reticulate* extract in RAW 264.7 macrophage cells. Food Chem Toxicol 2007, 45: 1545–1550.

[39] Ibrahim MA; Kainulainen P; Aflatuni A; Ikkala KT; Holopainen JK. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. Agric Food Sci 2001, 10: 243–254.

[40] Zayed AA; Saeed RMA; Namaky AH; Ismail HM; Mady HY. Influence of *Allium sativum* and *Citrus limon* oil extract and bacillus *thuringiensis Israelensis* on some biological aspects of Culex pipiens larvae (Diptera: Culicidae). W. J. Zool. 2009, 4: 109.

[41] Balasundram N; Sundram K.; Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem 2006, 99: 191–203.

[42] Mukherjee PK; Kumar V; Mal M; Houghton PJ. Acetylcholinesterase inhibitors from plants. Phytomedicine 2007, 14: 289–300.

[43] Menichini F; Tundis R; Loizzo MR; Bonesi M; Marrelli M; Statti GA; Menichini F; Conforti F. Acetylcholinesterase and butyrylcholinesterase inhibition of ethanolic extract and monoterpenes from *Pimpinella anisoides* V Brig. (Apiaceae). Fitoterapia 2009, 80: 297–300.

[44] Quintans J SS; Menezes PP; Santos MRV; Bonjardim LR; Almeida J RG S; Gelain DP; Araújo AAS; Junior LJ. Improvement of *p*-cymene antinociceptive and anti-inflammatory effects by inclusion in β-cyclodextrin. Phytomedicine 2013, 20: 436–440.

[45] Jiang S; Li J N; Jiang Z. T. Inclusion reactions of β-cyclodextrin and its derivatives with cinnamaldehyde in *Cinnamomum loureirii* essential oil. Eur Food Res Technol 2010, 230: 543–550.

[46] Fernandes LP; Oliveira WP.; Sztatisz J; Szilágyi IM; Novák C. Solid state studies on molecular inclusions of *Lippia sidoides* essential oil obtained by spray drying. J Therm Anal Calorim 2009, 95: 855–863.

[47] Silva FC. Available in: http://www.portaldosfarmacos.ccs.ufrj.br/resenhas_ciclodextrinas.html. Accessed on October 15 2016.

[48] Feitosa CM; Cavalcante AN; Chaves SKM; Araujo LS. Medicinal plants of Brazil and Alzheimer's disease: evolution in traditional use and pre-clinical studies. Asian J Biom Pharm Sci 2016, 6: 01–13.

IntechOpen