



## Study of anticholinesterase activity of the plant *Campomanesia lineatifolia* (myrtaceae)

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### Abstract

The study of anticholinesterase activity is of great importance, mostly, in the prevention of the hardly of Alzheimer. Being this way, o present work has as goal evaluate the inhibitory activity of ethanolic extract, hexane and dichloromethane of the leaves *Campomanesia lineatifolia* forward the enzyme acetylcholinesterase (AChE). The used leaves were collected in the Mocaminho Environmental Park in the city of Teresina-PI, from August 2010. They were triturated, ground and extracted exhaustively four times using the hexane solvent, diclorometano and etanol 99% for 16 days. The fractions were submitted to the activity inhibition test of the anticholinesterase enzyme (AChE). The tests showed that the plant presents inhibitory capacity of anticholinesterase (AChE) with o appearance of an inhibition halo of 6,0 mm for the hexane and dichloromethane extract and of 9,0 mm for the ethanolic extract.

Key words: Anticholinesterase, *Campomanesia lineatifolia*, inhibition

## Introduction

Countless vegetable species have medicinal interest, among them the gender plants of *Campomanesia*, belonging to the family Myrtaceae Jussieu divided into two subfamilies: Leptospermoideae and Myrtoideae, representing the two centers of geographic dispersion of the family. The species *Campomanesia lineatifolia* is represented by 140 genera, with more than 3.000 species distributed in the tropical and belonging subtropical regions to subfamilia Myrtoideae.<sup>(1-2)</sup>

Some species of the genus *Campomanesia* are native of the region southeast and Brazil's South, Uruguay and Argentina (*Campomanesia xanthocarpa* and *Campomanesia reticulata*) and another of bioma Closed (*Campomanesia adamantium* and *Campomanesia pubescens*), presenting as main difference o size.<sup>(3)</sup>

The leaves of the genus *Campomanesia* have medicinal properties as anti-inflammatory, antidiarrheal and antiseptic of the urinary ways, besides using in the influenza and regulates treatment of the intestinal functions. Used in traditional medicine as an infusion to treat gastrointestinal disorders such as diarrhea and stomach pains and for the regulation of intestinal flow and treatment of urethritis and cystitis.<sup>(4)</sup>

Alzheimer's Disease (DA) is a degenerative neurological disorder that attacks million people all over the world and the anticholinesterase substances are important in the treatment. The utilization of medicinal plants is demonstrated with success in developed researches.<sup>(5)</sup>

The substances that act in the enzyme acetylcholinesterase inhibition (AChE) are the most commonly used, like Donepezila, in the treatment of Alzheimer's disease. Alzheimer's Disease is responsible for the cognitive and behavioral dysfunctions and reaches mostly people with more than 60 years, being every time larger o number of people with this disease.<sup>(6)</sup> Researches developed with regard to the acetylcholinesterase inhibitors revealed that an acetilcolina levels increase (ACh) can

improve the cognitive problems in the early stages of DA.<sup>(7)</sup>

In the literature does not present studies related to the anticholinesterase activity of the *Campomanesia lineatifolia*, thus this work had for goal evaluate the inhibitory activity of the enzyme acetylcholinesterase for the ethanolic extract, hexane and dichloromethane through acetylcholinesterase inhibition assay by thin layer chromatography (TLC).

## Material and methods

### Obtainment of the plant material

The plant leaves *Campomanesia lineatifolia* were collected in Park Environmental Mocaminho, in the city of Teresina-PI, during august 2010.

### Extracts preparation

The leaves were droughts the ambient temperature for four days and after triturated and ground in knives mill. Were extracted exhaustively four times using the solvent hexane, dichloromethane and ethanol 99% for 16 days. The materials were dissolved partially filtered and concentrated in a rotary evaporator under reduced pressure and determined the dry weight of each extract. The obtained vegetable samples were submitted to the inhibition acetylcholinesterase activity test (AChE).

### Acetylcholinesterase Inhibition Test into thin layer chromatography (TLC).

O enzymatic rehearsal in TLC is a qualitative test. The following solutions were prepared : 50 mM Tris-pH 8; 3 mM of acid 5,5'-ditiobis-[2-nitrobenzóico] (DTNB or Ellman's reagent); 10 mM of acetylcholine iodide (ACTI); 1 mM of acid 5,5'-ditiobis-[2-nitrobenzóico] (DTNB) and 1 mM of acetiltiocolina iodide (ACTI). The enzyme AChE was lyophilized and dissolved in the solution of 50 mM Tris-pH 8. It let-if the enzyme in the solution for 10 minutes and afterwards for obtainment of a homogeneous

solution proceeded-if to the agitation for one more period 10 minute.

In this essay, we use the solution of the acid reagent 5,5 '-ditiobis-2-nitrobenzoic (DTNB) and acetylcholine iodide (ATCI) in tampon and, afterwards, applies-if the enzyme AChE. In the acetylcholinesterase inhibition rehearsal followed-if Elmann's Methodology, adapted by Rhee.<sup>(8)</sup> It applied-if small samples ethanolic extract of, dichloromethane and hexane using capillary tubes in TLC, Silicagel 60 F254, eluted in chloroform / methanol (9:1) and droughts the ambient temperature (Figure 1).

Figure 1: Representation of the application of the ethanolic extracts, dichloromethane and hexane in chromatographic plate. The black dots represent the application of extracts and positive control.

The chromatographic plate was sprayed with the stock solution of the AChE enzyme. For enzyme incubation, the plate was put under a support inside an exhaustion chapel and kept by 10 min. Past that time, the observation of a white halo around of the samples indicates the presence of enzyme acetylcholinesterase inhibitors. The caffeine was used as positive standard.

## Results and discussion

Extracts of medicinal plants can contain substances bioativas, where we include the substances that inhibit enzyme AChE. The elevation of acetylcholine through the enzyme inhibition AChE has been accepted for treatment against Alzheimer's Disease.<sup>(9)</sup> Thus, AChE inhibitors have become the remarkable alternatives in treatment against Alzheimer's.

Regarding the main biological activities of medicinal plants, some can is related to inhibition of AChE enzyme. There are an increasing by the research in the search for new inhibitors in substances originating from natural products, with special interest in the isolation and identification of new AChE inhibitors.<sup>(10)</sup>

For test acetylcholinesterase activity inhibition (AChE), the results showed inhibitory capacity of the acetylcholinesterase (AChE) with the appearance of an inhibition halo of 6,0 mm for the hexane extract and dichloromethane and of 9,0 mm for o extract ethanolic (Table 1).

see Table 1.

The analysis of the obtained results in this research can contribute for the elucidation of therapeutic options for the treatment of Alzheimer Disease through new acetylcholinesterase inhibitors. However, it is necessary the isolate the leaf constituents of *C. lineatifolia*, in order to identify which substance owns inhibitory activity against AChE and developing studies with regard to the bioactive principles with o purpose of certifying this activity.

## Conclusion

O study and qualitative tests as the rehearsals into thin layer chromatography allowed the detection of anticholinesterase activity of bioactive compounds contained in the extracts hexane, dichloromethane and ethanolic of the plant leaves *Campomanesia lineatifolia*, showing-if very promising for the isolation and characterization of compounds acetylcholinesterase inhibitors (AChE), that can assist in the treatment of the hardly of Alzheimer.

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Extract of the <i>C. lineatifolia</i>	Part of the Plant	Sample concentration (mg/mL)	Inhibition of the Acetylcholinesterase (mm)
Hexane	Leaf	5mg/mL	6
Dichloromethane	Leaf	5mg/mL	6
Ethanol	Leaf	5mg/mL	9

Table 1: Results of inhibition of the acetylcholinesterase enzyme (AChE) in TLC for the extracts from *C. lineatifolia*