



Communication/Comunicação

Acetylcholinesterase inhibition starting from extracts of *Bauhinia variegata* L., *Bauhinia var. candida* (Aiton) Buch.-Ham., and *Bauhinia unguolata* L

Inibição de acetilcolinesterase a partir de extratos de *Bauhinia variegata* L., *Bauhinia variegata var. candida* (Aiton) Buch.-Ham., e *Bauhinia unguolata* L

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ABSTRACT

Introduction: A treatment to the Alzheimer's disease consists inhibition of the acetylcholinesterase, which is responsible for the acetylcholine control in the synapses. **Methods:** We have investigated the potential of inhibition of the acetylcholinesterase produced by hexane extracts of leaves, branches, and flowers from three *Bauhinia* specimens, which is based on the technique of thin layer chromatography and on identifying the organ of the plant that possesses larger concentration of inhibitors. **Results:** Retention factor analysis shows values of 0.31aA, 0.31aA, and 0.46aB for flowers *B. variegata*, *B. var. candida*, and *B. unguolata*, respectively. **Conclusions:** The flower extract of *B. unguolata* is the most suitable for further studies on this inhibition.

Keywords: Acetylcholinesterase. *Bauhinia*. Chromatography.

RESUMO

Introdução: Um tratamento para a doença de Alzheimer consiste na inibição da acetilcolinesterase, responsável pelo controle de acetilcolina nas sinapses. **Métodos:** Nós investigamos o potencial de inibição da acetilcolinesterase produzido por extratos hexânicos de folhas, ramos e flores de três espécimes de *Bauhinia*, realizado a partir da cromatografia em camada delgada e que órgão possui maior concentração de inibidores. **Resultados:** Os fatores de retenção apresentaram 0,31aA, 0,31aA e 0,46aB para as flores de *B. variegata*, *B. var. candida* e *B. unguolata* respectivamente. **Conclusões:** O extrato de flores de *B. unguolata* é o mais satisfatório para futuros estudos desta inibição. **Palavras-chave:** Acetilcolinesterase. *Bauhinia*. Cromatografia.

Alzheimer's disease (AD) has reached about 20 million people worldwide, and this index is only set to grow due to the increasing life expectancy that has been observed in recent years. This disease is characterized by affecting the short-term memory and associative cortical areas; thus, the carrier of AD needs permanent care and stress, psychological, and financial support from family caregivers¹.

It is believed that the pathophysiology of AD is related to the presence of senile plaques and neurofibrillary tangles, the bead-vacuolar degeneration, and neuronal loss. At cellular levels, AD is related mainly to a decrease of acetylcholine (ACh) in the synaptic cleft, so several cholinomimetic therapies have been tested for the treatment of AD; however, more effective effects could be verified by treatments that lead to the inhibition of acetylcholinesterase (AChE)².

The use of medicinal plants is one of the oldest practices carried out by man and is still very widespread today. Currently, many people and health professionals choose to perform therapies with these plants as complements to the treatments based on synthetic³. The species of the genus *Bauhinia*, endemic to cerrado biome, are used by the population and are being considered primarily as antihyperglycemic adaptogenic. Known as Pata-de-vaca (Orchid tree), these species are also found in street trees and have compounds that are interesting medicinally⁴. Due to these characteristics, these species represent examples that have the potential inhibition of acetylcholinesterase, and thus, according to the results, these could assist in the treatment of AD.

We collected leaves, flowers, and branches of *Bauhinia unguolata*, *Bauhinia variegata*, and *Bauhinia variegata candida* from single trees in the following locations: at the Jovelino Rabelo street, next to number 530 in Porto Velho, Divinópolis, MG; at the Jovelino Rabelo street, next to number 530 in the same neighborhood in Divinópolis, MG; and at the Maestro João Pinto street, in the home of number 85 in the Esplanada in Divinópolis, MG, Brazil.

The samples were washed, chopped, and dried in an oven at 45°C for 5 days. They were then crushed in a blender⁵. These samples were also prepared to be the dried specimens and subsequently deposited in the herbarium of the Universidade José do Rosário Vellano (UNIFENAS), Divinópolis campus; identified by Professor Doctor Guilherme Araújo Lacerda CRBio 44480/04-D; and cataloged by the numbers: *Bauhinia unguolata* L. 024, *Bauhinia variegata* L. 025, and *Bauhinia variegata candida* (Aiton) Buch-Ham 026.

According to technique adapted from Souza⁶, 116g of each dried sample were added to 1L of hexane in glass jars capped and covered with brown paper. The flasks were shaken mechanically for 10 min and were then left to stand for 72h for maceration. The macerate was vacuum-filtered, and the solvent was extracted by distillation and water-bathed at 60°C.

For the analysis of acetylcholinesterase inhibition, thin layer chromatography (TLC) was used as recommended by Moraes², with some adaptations.

The TLCs were performed in duplicate. In each plate of silica gel, were applied to extracts of species of *Bauhinia*, grouped according to plant part investigated. Then the plates were developed in the system n-hexane-acetone (80:20) in tanks previously saturated until 0.5cm of silica final. After the run, the plates were air-dried and received nebulized solution of N-bromosuccinimide. Excess oxidant was removed under a stream of hot air for 20 min, and then, the solution was nebulized with cholinesterases. After 20 min, the

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solution of 2-naphthyl acetate was sprayed with fast blue B, and the chromogenic analysis was performed after 30 mins. The results were recorded from photographs on graph paper. The retention factor was calculated in places where there was the appearance of white spots.

For the statistical analysis of retention factors, the experiment employed a completely randomized design with two replications, using the software Sisvar[®] according to Ferreira⁷. The factorial schemes used were the three species and three plant parts studied. For the retention factor, we used the t-test to detect differences between treatments and the t-student (LSD) (p-value<0.05) for comparison of means.

From the analysis of chromatography, it was possible to detect the presence of white smudges for this purpose, which represents qualitatively the inhibition of acetylcholinesterase, as the methodology used was based on the cleavage of 2-naphthyl acetate by acetylcholinesterase to form the 2-naphthol, which reacts with Fast Blue B to generate the diazonium purple coloration². The presence of enzyme inhibitors was observed mainly in flowers (Figure 1), but they were also detected with lower intensity on the branches (Figure 2). The leaves showed no inhibitory action of the enzyme (Figure 3).

The flowers are both sinks of organic compounds on macro and micronutrients⁸, and it is well known that the secondary compounds, potential inhibitors of acetylcholinesterase, physiologically act in plant defense against herbivores, as attractive to pollinators, and hormones used between individuals of the same species for different purposes³.

These factors have great relevance in an attempt to justify the higher prevalence of secondary compounds in the flowers, as in this experiment, the samples were collected during the flowering season of the species of *Bauhinia*. As the branches are the aerial structure of a tree, they have the lowest concentration of micronutrients and secondary compounds because they play major roles in the support,

transport, and storage of the leaves, flowers, and roots⁹. In this context, it should be argued that the enzyme inhibitors reported in the business are possibly present in this structure being in the transport process.

Studies have shown that the values of secondary compounds can vary significantly between mature leaves and young leaves; the latter has shown higher concentrations of the compounds, and this happens

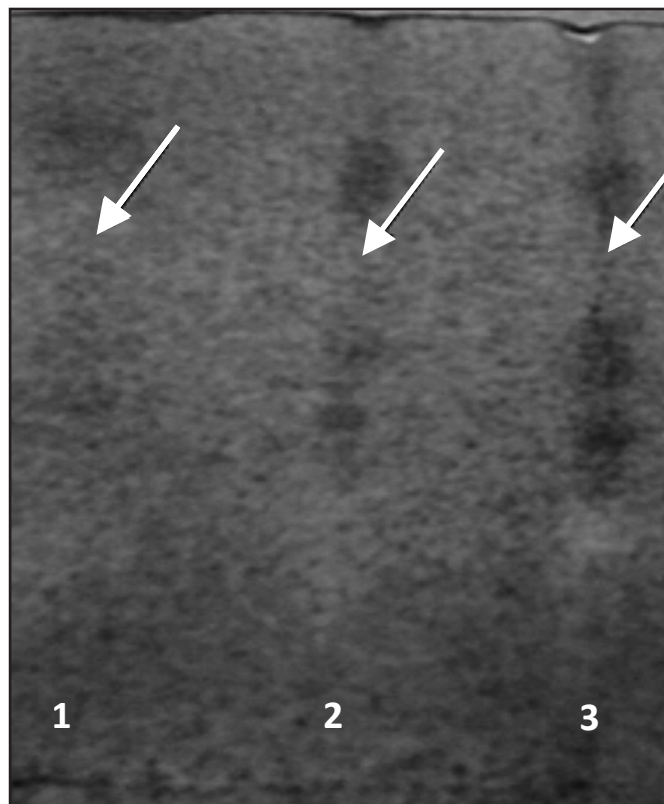


FIGURE 2 - Inhibition of acetylcholinesterase from extracts of stems: *Bauhinia unguolata* (1), *Bauhinia variegata* (2), and *Bauhinia variegata candida* (3)

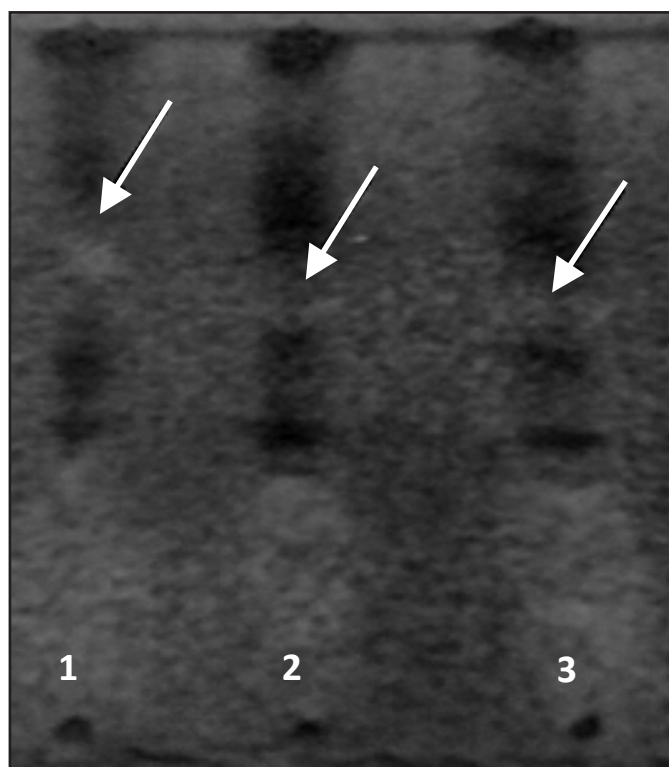


FIGURE 1 - Inhibition of acetylcholinesterase from extracts of flowers: *Bauhinia unguolata* (1), *Bauhinia variegata* (2), and *Bauhinia variegata candida* (3).

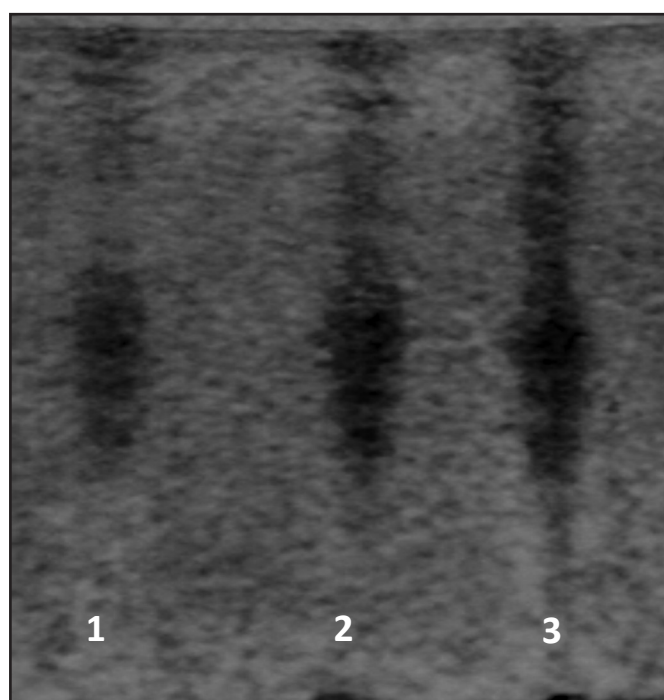


FIGURE 3 - Inhibition of acetylcholinesterase from extracts of leaves: *Bauhinia unguolata* (1), *Bauhinia variegata* (2), and *Bauhinia variegata candida* (3)

due to translocation of substances to other tissues as growth leaves¹⁰. When collecting leaves, we selected those with the larger aim, and we believe that this choice may have influenced the negative results reported in the leaves.

Shown in **Table 1** are the means of the retention factors of the species surveyed. Based on the interpretation of the t-student (LSD), statistical values do not differ to a nominal level of 5% within the body between the species. However, there is an increasing statistical difference between the Rf for the organs of *B. unguolata*.

TABLE 1 - Retention factor (Rf) calculated between specimens and between vegetable organs of *Bauhinia*.

| Plants | Retention factor (Rf) | | |
|-----------------------------------|-----------------------|----------|---------|
| | leaves | branches | flowers |
| <i>Bauhinia variegata</i> | 0.00 aA* | 0.12 aA | 0.31 aA |
| <i>Bauhinia variegata candida</i> | 0.00 aA | 0.26 aA | 0.31 aA |
| <i>Bauhinia unguolata</i> | 0.00 aA | 0.12 aA | 0.46 aB |

Note: *Means followed by the same lowercase letters in the column and uppercase letters on the line do not differ by t-student (LSD) at a nominal level of significance of 5%.

Currently, it is known that the genus *Bauhinia* has mainly alkaloids and phenolic compounds in its constitution¹⁰. Even some studies on cognitive function, related to inhibiting the enzyme acetylcholinesterase are the alkaloids, several studies have also been emphasized to the flavonoids and saponins, because mainly tonic properties stimulant, purifying and immune atioxidante as treatments for important degenerative disorders.

It is, therefore, suggested that in-depth reviews of the chemistry of the flowers of *Bauhinia unguolata* be performed to elucidate the secondary compounds and to quantify the inhibition. From the results of the statistical analysis, we conclude that the extract of the flowers of *Bauhinia unguolata*, taken within the flowering period, is the most suitable to serve as a basis for further studies on this inhibition.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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